MOVEMENTS OF THE SUBTALAR AND TRANSVERSE TARSAL JOINTS

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SIX FIGURES

The articulations joining the talus, calcaneus, navicular and cuboid bones present a complicated arrangement. There are, however, only two functional joints in this region: the subtalar, or lower ankle joint, comprising all the articulations of the talus below the ankle joint, and the transverse tarsal joint, formed by the articulations of the talus and the calcaneus with the navicular and the cuboid bones. These joints link together the bones which form the middle portion of the longitudinal arch. Their movements are not extensive, but they play an important part in the mechanical function of the foot. For an understanding of the true action of these joints, measurements of their movements and determinations of the axes of their rotations are needed.

METHODS

The feet used in this investigation were obtained from cadavers. All were of normal appearance from adult, white males. They were removed at the ankle joint and freed of their large muscles and tendons. After this preparation the feet were placed in a glycerine solution to keep them soft and pliable.

No special attempts were made to measure the extent of movement in the joints, since the reliability of such measurements, when made on cadavers, is questionable. Although the range of joint movement may depend on the state of muscles and ligaments, the character of the movement is regulated

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by the shape of the articulation. As long as the articular surfaces remain congruent, the directions of the movements obtained in the joints of the cadaver are probably comparable to those in the natural foot.

The relative motion occurring between two bodies may be analyzed by holding one body stationary and observing the path traveled by the other. Selection of the fixed member is optional, for the motion will be the same, except for direction, regardless of which part is immobilized. In the present case the calcaneus has been immobilized, and the motion of adjacent parts has been referred to the calcaneus as if it were a fixed body.

The first step taken in measuring the motion of a joint, such as the subtalar joint, was to determine the position of the plane in which the movement is carried out. The foot was firmly clamped at the calcaneus and three long rods were rigidly attached to the talus. The tips of these rods, arranged in tripod fashion, form the corners of a broad triangular plane which moves with the talus about the subtalar joint. As long as the plane is situated oblique to the plane of movement for the joint, turning of the joint will cause it to tilt. By adjusting the corners of the plane, one position can be found in which it remains flat, rotating in its own plane as the subtalar joint is moved. When it is in this position, the three cornered plane coincides with, or lies parallel to, the plane of rotation of the joint. The greater the area of the trial plane, the more accurate will be the determination of the plane of rotation. With a triangular plane having sides 50 cm. long, a deviation of 2 or 3 degrees from the plane of rotation of the joint is discernible.

It is difficult to make accurate observations of the short paths followed by points located on the surface of a bone when the movement of that bone is limited to a rotation of but 10 or 15 degrees. An arc of much longer radius can be obtained by attaching a pointer to the bone with its tip directed outward from the axis of rotation. Such a pointer, when rigidly fixed to the bone, performs motion having all

the characteristics of the motion of the body to which it is attached. A method for fastening several pointers to the talus is illustrated in figure 1. In this arrangement a $\frac{1}{4}$ inch rod, bolted through the body of the talus, supports a cork to which steel needles are attached.

If the pointers connected to the talus are to move in horizontal planes, the foot must be oriented so that the plane of

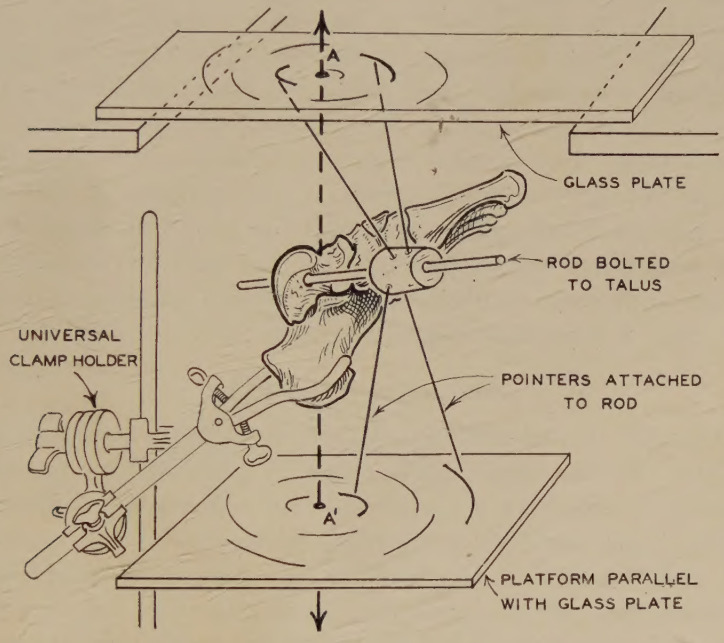

Fig. 1 Apparatus used in determining joint axes. Concentric arcs are traced on two platforms as the talus is turned at the subtalar joint. AA" indicates the position of the axis of the joint.

rotation of the joint is horizontal. The foot was clamped in such a position; then it was placed between two level platforms (fig. 1). The tips of the pointers were adjusted so that they just touched the upper or the lower horizontal plane, and the course of their motion was observed as the talus turned about the subtalar joint. Each pointer traces out a path on one of the planes which may be marked in

pencil. By moving the pointers to different locations, a series of these paths of motion can be traced on the two planes. When this was done, the paths were found to be arcs, arranged concentrically about a center point at which no movement of the pointers occurred. Thus two points denoting centers of rotation were located, one in the upper, the other in the lower plane. A line connecting the two points establishes the axis of the joint.

Two planes of reference were used to define the position of the joint axis in relation to the foot. The first plane passes vertically through the foot along its longitudinal axis from the middle of the heel to the space between the first and second toes. The second plane is a horizontal base plane formed by the sole of the foot. A projection of the joint axis was made on each plane, and the angle of slope of the axis projection was measured. In this manner the position of the joint axis is obtained as it would appear in a side view and in a dorsal view of the foot.

THE SUBTALAR JOINT

Although it includes several separate articular surfaces, the subtalar, or lower ankle, joint is regarded as an entity because it comprises a functional unit. Movement performed at this joint involves a rotation between the talus on one side and the rest of the foot on the other. When the talus is fixed, this action produces a combination of eversion, abduction and slight dorsi-flexion of the foot about the talus, or, more briefly, pronation. In the reverse direction, the action is termed supination. The approximate position of the axis for the rotatory motion has been given by Henke (1859), Meyer (1886), Dönitz ('03), R. Fick ('11) and by Elftman and Manter ('38) who all agree, in a general way, that it passes through the head of the talus and extends from below on the lateral side of the heel, upward, forward and medially.

The results of measurement of the axis of the subtalar joint are given in figure 2 which shows its average location in sixteen feet. The position of the axis agrees with the ob-

servations which others, using less accurate methods, have reported. Considerable variation occurred in individual feet. The medial slant of the axis varied between extremes of 8 and 24 degrees, while the amount of vertical tilt varied between 29 and 47 degrees.

Screw-like character of the joint. The plane of rotation of the subtalar joint was found, upon measurement, to lie on a slant with respect to the axis. Consequently the movement of the joint consists of rotation in a direction oblique to the axis of the joint. R. Fick ('11) has demonstrated that movement in a plane oblique to an axis is physically limited to

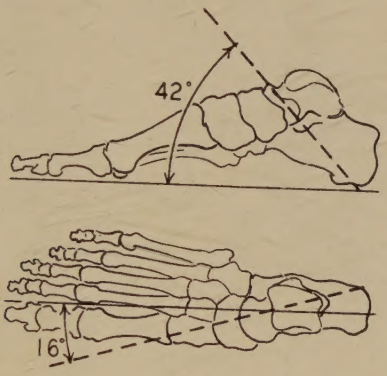

Fig. 2 Position of the axis of the subtalar joint in the foot (dotted line). Projection on a vertical plane above, on a horizontal plane below.

either a translatory sidewise slipping, or to screw-like rotation, providing the congruity of the two surfaces is maintained. Since sidewise slipping does not occur, the action of the subtalar joint is very likely that of a screw.

In figure 3 a comparison is made between the posterior calcaneal facet, upon which the talus rests, and the surface of a screw. A point *o* following the surface of the screw will move (momentarily) in the horizontal plane *hh'*. This plane makes an angle *s* with the plane *tt'* which is drawn perpendicular to the axis of the screw. The angle *s*, called the helix angle, is a measure of the slope of the helix of the

screw and its size depends on the screw's pitch. It is not necessary to measure the angle s directly to obtain the helix angle of the subtalar joint. Another angle, which is more conveniently measured, may be constructed equal to s. A perpendicular pp' dropped from the axis of the joint forms an angle s' which is equal to the helix angle s. From measurements of this angle, the average helix angle for the subtalar joint was found to be 12 degrees. In terms of pitch, or thread interval, this is equivalent to 0.67 inch for a screw 1 inch in diameter.

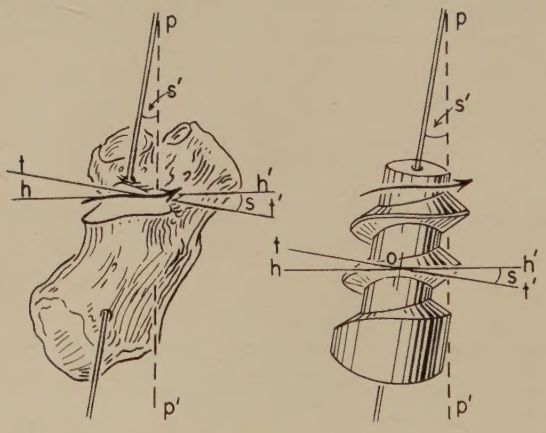

Fig. 3 Comparison of the posterior calcaneal facet of the right subtalar joint with a right-handed screw. Arrow represents the path of a body following the screw. hh' is the horizontal plane in which motion is occurring. tt' is a plane perpendicular to the axis of the screw. s is the helix angle of the screw, equal to the angle s' which is obtained by dropping a perpendicular pp' from the axis.

Further proof of the screw-like character of the subtalar joint is obtained by a study of the geometry of the posterior calcaneal facet, the principal surface on which the talus moves. Serial sections of the calcaneus made perpendicular to the joint axis reveal that the contours cut from this articular surface form spiral rather than circular arcs. These spiral figures closely resemble segments of a Spiral of Archimedes, which is the figure obtained by cutting transverse sections through an oblique helicoid, or screw-shaped surface.

The remaining articular surfaces of the subtalar joint, formed by the middle and anterior calcaneal facets, are also suitably shaped to allow screw-like action. They form a shallow trough upon which the talus is able to slide forward or back as well as to rotate.

The subtalar joint behaves like a right-handed screw in the right foot and like a left-handed one in the left foot. When the right calcaneus is held stationary, pronation causes the right talus bone to turn clockwise and, at the same time, to advance along the joint axis. Forward displacement of the talus is very small. If it is assumed that the head of the talus lies 2 cm. from the joint axis, its forward displacement would be approximately 1.5 mm. for each 10 degrees of rotation in the subtalar joint.

THE TRANSVERSE TARSAL JOINT

The transverse tarsal joint (Chopart's) includes the cup-shaped articulation of the navicular with the head of the talus and the saddle-shaped articulation between the cuboid and the calcaneus. The cuboid and the navicular, along with the anterior part of the foot, form a unit which moves upon the calcaneus and the talus. The nature of the movement which occurs, and the axis about which it centers have received widely varying interpretations. Meyer (1886) and Fick ('11) were the chief proponents of a vaguely located, "horizontal" axis running lengthwise through the foot. Elftman and Manter ('38) proposed a different axis, more nearly transverse, which corresponds with the axis of this joint in the chimpanzee.

Two separate movements of rotation have been consistently obtained in the transverse tarsal joint and two different axes have been measured. One axis is the center of a pivotal movement of the cuboid upon the calcaneus; the other axis, placed obliquely in the foot, denotes the center of rotation as the broad concavity of the cuboid slides upon the adjoining surface of the calcaneus. Figure 4, a view of the posterior surfaces of the navicular and cuboid bones, illustrates the

action of these articular surfaces in carrying out either type of movement. The shapes of the joint surfaces allow either movement to occur without loss of a well matched fit with the corresponding surfaces of the talus and the calcaneus.

First axis. The results of measurement of the first axis in nineteen feet are given in figure 5. The axis extends lengthwise through the foot, but it is not placed horizontally. Instead, it slopes upward in front at an angle of 15 degrees. The axis also diverges slightly toward the medial side of the foot. When the calcaneus is fixed, movement in the trans-

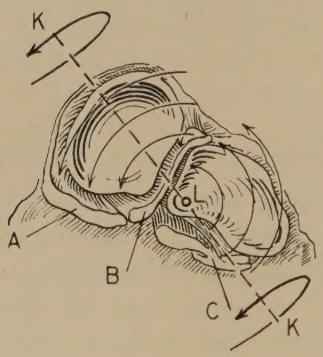

Fig. 4 Posterior view of the right transverse tarsal joint showing the articular surfaces of the navicular and the cuboid. Light arrows indicate motion about a longitudinal axis, *L;* heavy arrows indicate motion about an oblique axis, *KK*. A, plantar calcaneo-navicular ligament; B, deep portion of the bifurcate ligament; C, long and short plantar ligaments.

verse tarsal joint consists mainly of eversion or inversion of the fore part of the foot. Because of the upward tilt of the axis of the joint, some abduction is associated with eversion and some adduction with inversion.

Movement about the longitudinal axis of the transverse tarsal joint involves screw-like action rather than simple rotation. When measured by the same method which was applied in measuring the subtalar joint, the helix angle for the transverse tarsal joint was found to be 10 degrees (average in nineteen feet measured). The direction of the screw is opposite to that of the subtalar joint being left-handed in

the right foot and right-handed in the left. Eversion of the right foot about the transverse tarsal joint is accompanied by counterclockwise rotation of the cuboid and the navicular with a small advancement of these bones along the axis of the joint.

Combined movements of the subtalar and transverse tarsal joints. The contention has frequently been made that movements of the subtalar and transverse tarsal joints are interdependent and that action in both joints occurs simultaneously. When both joints are allowed normal freedom, it can be observed that pronation in the subtalar joint is usually accompanied by eversion of the foot at the transverse tarsal joint. R. Fick ('11) demonstrated that movement of the transverse tarsal joint was greatly restricted when the subtalar joint was immobilized by fastening the talus to the calcaneus. Even more significant is the limitation of movement in the subtalar joint which results when the navicular bone is rigidly fastened to the calcaneus. Ordinarily, the screw-like action of the talus causes the head of the talus to be carried forward in pronation pushing the navicular bone ahead of it. When this displacement of the navicular is prevented, little movement can be obtained in the subtalar joint. Since the mechanical restriction of either of the two joints has a marked effect on the mobility of the other, neither one can be said to be independent of the other. Under normal circumstances both joints act together.

Many years ago Henke (1859) suggested a modified screw-like arrangement for the tarsal joints. The analysis of the mechanism of these joints which is presented here also involves the action of screws; otherwise it differs entirely from Henke's hypothetical mechanism. In the right foot, eversion about the transverse tarsal joint turns the navicular bone counterclockwise with respect to the calcaneus; moreover it moves this bone forward along the axis of rotation in the manner of a left-handed screw. Such a displacement of the navicular releases the head of the talus and allows it to advance. This movement is brought about by pronation of the talus at the

subtalar joint. The talus undergoes clockwise rotation with respect to the calcaneus, and the head of the bone advances along the joint axis by screw-like action which, in this case, is right-handed.

The subtalar and transverse tarsal joints may well be compared to dual screws whose members are connected by the contact of the talus with the navicular. The screws involved have opposing pitches and turn in opposite directions. Rotation of one member will be transmitted to the other in much the same manner as one gear is turned by another through the contact of an individual gear tooth.

The extent of rotation of the two joints is limited in the foot. The small range of movement provided for by the articular surfaces is placed under further restriction by the action of ligaments and muscles. Some of these ligaments and muscles lie at the sides of the joint axes, but the strongest ones are in the plantar region of the foot. Because of their position, these plantar muscles and ligaments tend to oppose forward displacement of the navicular bone. This appears to be an effective means of restricting pronation and eversion of the foot.

The posture of the foot as a whole is considerably changed by combined movements of the two main tarsal joints, but these readjustments have little effect on the conformation of the longitudinal arch. Pronation in the subtalar joint causes abduction and dorsi-flexion of the whole foot with respect to the talus. Eversion in the transverse tarsal joint lowers the medial border of the navicular bone a few millimeters and flattens the medial border of the arch slightly. The great toe lies directly below the axis of the transverse tarsal joint, and it is moved laterally upon eversion. The outer toes are farthest from this axis of rotation, and they undergo a more extensive displacement outward and upward.

Second axis. A second type of movement of the transverse tarsal joint is generally obtainable. The action produced is one of combined dorsi-flexion and abduction of the fore part of the foot while the talus and the calcaneus remain fixed.

Reversal of the movement produces plantar-flexion and adduction. The axis for this movement is steeply inclined vertically, and, at the same time, slanted obliquely across the foot. Its average position, measured in fifteen feet, is denoted in figure 6. No indication of screw-like action was found in making these measurements.

Movement about the second axis of the transverse tarsal joint is notably variable in range both in cadavers and in the natural foot. In cadavers, restriction of movement in the

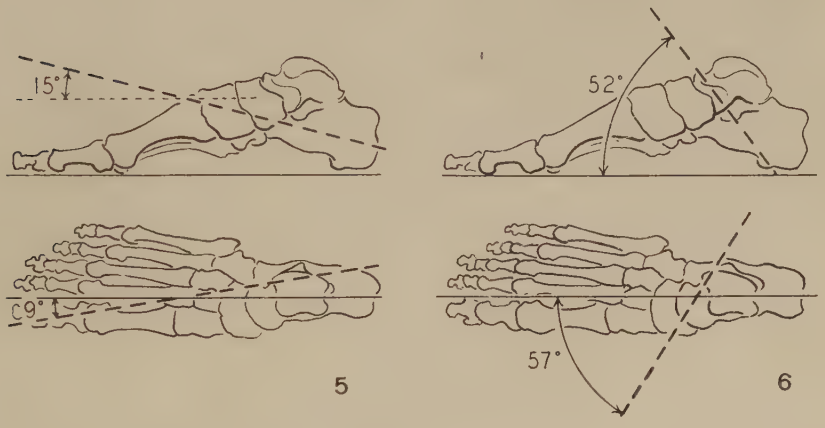

Fig. 5 Projections of the longitudinal axis of the transverse tarsal joint (dotted line) showing its position in the foot.

Fig. 6 Projection of the oblique, or transverse, axis of the transverse tarsal joint to show its position in the foot.

joint can be released by cutting certain ligaments. Three groups of thick ligaments occupy suitable positions for checking dorsiflexion of the foot at the transverse tarsal joint. These are: (1) the plantar calcaneo-navicular ligament; (2) the long plantar and the calcaneo-cuboid (short plantar) ligaments; and (3) the bifurcate ligament. Transection of any of these ligaments will increase the range of joint movement. Cutting the deep portion of the bifurcate ligament (calcaneo-navicular portion) was found to be particularly effective in freeing the joint.

Relationship to flatfoot. Movements of dorsi- or plantar-flexion in the transverse tarsal joint are associated with pronounced changes in the longitudinal arch of the foot. The axis of the joint is comparable to a hinge placed in the middle of the arch. When the fore part of the foot is dorsi-flexed and abducted, the arch is flattened; when it is plantar-flexed and adducted, the arch is raised. In normal feet the range of motion is small and the degree of flattening or raising of the arch is accordingly slight. Extreme dorsi-flexion at the transverse tarsal joint may, however, produce a foot posture resembling flatfoot, as Elftman and Manter ('38) have suggested. If dropping of the arch does involve progressive assumption of a dorsi-flexed position at the transverse tarsal joint, abduction of the fore part of the foot should also be expected, for the action of the joint is such that abduction and dorsi-flexion proceed together. In describing flatfoot, Morton ('24) has called attention to the abduction of the fore part of the foot which occurs early in the development of this disorder. In advanced cases, abduction may amount to as much as 30 degrees.

A normal foot could be transformed into a flat-arched foot by movements in the subtalar and transverse tarsal joints. The order of these changes might be as follows: (1) Pronation in the subtalar joint accompanied by eversion of the transverse tarsal joint about its longitudinal axis with forward displacement of the navicular bone; (2) widening of the gap between the navicular and the sustentaculum tali and stretching of the plantar ligaments which bridge this space; (3) increased mobility of the transverse tarsal joint about its oblique, or transverse, axis due to laxity of the plantar ligaments;[1] (4) gradual assumption of a position of dorsi-flexion and abduction about the oblique axis of the transverse tarsal joint with flattening of the longitudinal arch. No shifting of joint axes is involved in this process.

[1] If postural tonus of the musculature is, by itself, capable of controlling the joints, it must be assumed that the muscles, also, become less efficient.

The movements have the same directions as those regularly present, but their extent is increased beyond normal limits. Without clinical study it is impossible to determine the extent to which these changes at the joints actually occur in dropping of the longitudinal arch.

SUMMARY

The locations of axes for movements of the subtalar and the transverse tarsal joints have been measured by new methods which provide for the determination of screw-like action as well as the designation of the axes.

Results show that the subtalar joint causes the talus to move as a right-handed screw in the right foot and as a left-handed one in the left foot. The transverse tarsal joint allows the fore part of the foot to rotate about a longitudinal axis moving like a screw directed opposite to that of the subtalar joint. Pronation at the subtalar joint is accompanied by eversion in the transverse tarsal joint while the two screws involved in the combined movements turn in opposite directions.

The transverse tarsal joint is capable of moving about a second axis which lies obliquely in the foot. In contrast to the small changes produced by movements about the other two axes, motion about this oblique axis causes a marked change in the conformation of the longitudinal arch.

By appropriate rotations about all three axes of the principal tarsal joints, the foot can be brought into a position similar to that seen in flatfoot.

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A STUDY OF QUADRUPLETS

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ONE FIGURE

INTRODUCTION

The relative infrequency of quadruple births, which have been shown by Diddle and Burford ('35) to be but one in every 654,455 labors, seems to justify the description of each new case. The quadruplets described in this paper are of particular interest since they were delivered and preserved with all fetal membranes intact. Further, these quadruplets are of interest inasmuch as they contribute to the evidence in favor of an hereditary influence in the occurrence of multiple human births.

The interest aroused by this set of quadruplets and the varied condition of the literature has prompted the author to collect and tabulate all available data pertaining to the frequency of quadruplets with regard to the number of ova involved in their production, the distribution of sex and the number of previous multiple births in families bearing quadruplets.

Clinical data

The mother, colored, age 26 years, had had three previous labors which resulted in two single births and one set of twins. The quadruplets were born, according to the attending physician, approximately 5 months after the last menstrual period. At this time the patient was suffering with pyelitis and the miscarriage occurred shortly after a severe chill.

The family history revealed that multiple births were quite common. The patient's great grandmother had had twins, her

maternal aunt had had twins, and the daughter of this aunt, who is this mother's cousin has had triplets. The father could recall no multiple births in his family.

Placentae and membranes

The placentae were three in number and consisted of one large and two small masses (fig. 1). Of the smaller placentae one (A) was oval, being about 145 mm. long and 75 mm. wide.

Fig. 1 A photograph of the fetuses showing the placental relations.

The other small placenta (D) was round and approximately 105 mm. in diameter. The large placenta represented the fused placentae of fetuses B and C. It was oval in shape, being 150 mm. long and 110 mm. wide.

All cord attachments were marginal except for A, which was central. The attachments of B and C were marginal for the individual placentae, but central for the fused placental mass.

Each small placenta was associated with a single fetus which was enclosed in its own chorio-amniotic sac. Two fetuses, B and C, were attached to the larger mass. Dissection of the membranes of B and C revealed that the wall separating the two fetuses consisted of four parts, i.e., two amnions and two chorions. Despite the close proximity of the adjacent chorions there was no indication of fusion and they were separable throughout. On the uterine surface of the placenta no division could be made. On the fetal side, however, the blood vessels could be seen to spread away from the adjoining placenta.

Fetuses

The four fetuses consisted of three females and one male (fig. 1). Two of the females (A and D) were associated with single placentae while one female (B) and one male (C) were attached to the fused placenta. Crown-rump and crown-heel measurements were made on all fetuses (table 1). The figures

TABLE 1

Length of the fetuses in millimeters.

	A	B	C	D
C-R length	125	128	144	120
C-H length	199	206	222	199

obtained from these measurements are only slightly above those given by Arey ('40) for fetuses of 4 months. An examination of the palmar and plantar surfaces of the hands and feet revealed that the epidermal ridges were not yet developed. Since these first appear during the eighteenth week (Pinkus, '10) it seems that the age of these quadruplets should be placed at approximately 17 weeks.

DISCUSSION

Fetal relationships as determined from a study of the fetal membranes indicate that these quadruplets were derived from four fertilized ova rather than from two or three ova. In either of the latter cases one would expect to find two or three

of the fetuses enclosed in a common chorion, depending upon the number of ova involved in their production.

Rossi ('26) studied eleven cases of quadruplets and found that about 18.2% were quadrazygotic. Of the eleven cases cited by Rossi and twenty-six additional cases not included in his study, 32.4% were found to be quadrazygotic (table 2). Other classes of quadruplets seem to be less frequent; monozygotic, 8.2%; dizygotic, 29.7%; and trizygotic, 29.7%. It should be noted that in approximately one-half of the cases included in this table the diagnosis was made upon the basis

TABLE 2

The frequency of quadruplets according to the number of ova.

	AUTHOR'S STUDY			ROSSI'S STUDY		TOTAL	
Source	No. ova	No. cases	%	No. cases	%	No. cases	%
Clarke Fallett	1	2	7.7	1	9.1	3	8.2
Diddle and Burford Dobis Häfeli Papatestas Rocher and Lafond	2	5	19.2	6	54.5	11	29.7
Constantine Dawson Harrisson Hermstein and Pfalz Lantuejoul and Reglade Pryor ('39) Sinclair Tartakoff White	3	9	34.6	2	18.2	11	29.7
Budd Fathergill Miller Pinard Pryor ('36) Roberts Stahl Wilking Williams Wilson	4	10	38.4	2	18.2	12	32.4
Total		26		11		37	

of fetal membranes alone and therefore may be subject to some error since Arey ('22) has shown that dizygotic twins may become monochorial as the result of fusion of the adjacent chorions.

Quadruplets classified upon the basis of sex fall into five groups as shown in table 3. In a total of 149 cases it was found that quadruplets composed of two males and two females comprise the largest group. It is of interest to note that the groups consisting of all males or all females were slightly larger than

TABLE 3

The frequency of quadruplets according to sex distribution.

	AUTHOR'S STUDY		ROSSI'S STUDY*	TOTAL	SEX DISTRIBUTION	
	Source	No. cases	No. cases	No. cases	Male	Female
Quadruplets of same sex	Constantine Fleming Lloyd Papatestas Pryor ('36)	5	25	30	4	—
	Clarke Diddle and Burford Häfeli Pendergraft Sinclair	5	26	31	—	4
Quadruplets of different sex	Dobis Dutt Gilmore Harrisson Loudon Wilson	6	18	24	3	1
	Dawson Fotheringham Miller Pryor ('36) Williams	5	21	26	1	3
	Hermstein and Pfalz Pinard Pryor ('39)	3	35	38	2	2
	Total	24	125	149	294	302

* Rossi's study includes figures from Brattström, Guzzoni and Veit.

those in which there were three of one sex and one of the other. Since only 8% of the quadruplets were found to be monozygotic it must be assumed that a majority of those quadruplets of the same sex were derived from more than one ovum.

The possibility of an hereditary factor being associated with the occurrence of multiple births has long been of interest. Danforth ('15) concluded that uniovular as well as biovular twinning seems to be transmitted through the female. He also points out the possibility of the transmission of the uniovular factor through the male. In 22 of 58 cases of quadruplets found by the author there was no family history given. Of the remaining 36 cases 14 definitely state that no previous multiple births were known in the family of the mother. In 21 cases, or 58.3% of all cases in which family histories were given, there were other multiple births (table 4). In six of these cases, however, the multiple births were limited to the mother of the quadruplets. With the exclusion of such cases it was found that in 41.6% of the cases multiple pregnancies were experienced by other members of the family.

In five cases (White, '10; Thompson, '22; Fotheringham, '27; Papatestas, '29, and Sinclair, '40) there were histories of multiple births among the antecedents of the father as well as the mother. In three cases (Hermstein and Pfalz, '31; Dobis, '36, and Gilmore[1]) there was a paternal but no maternal history. Since in all three of these instances more than one ovum was involved the causative factor could not have been introduced by the male.

In several instances the mother of the quadruplets was a twin (Brattström, '14; Constantine, '35; Fleming, '35, and Sinclair, '40). In one case (Sinclair) the mother was a monozygotic twin while in another (Brattström) dizygotic. In three cases the father had twin sisters or brothers (Hermstein and Pfalz, '31; Dobis, '36, and Sinclair, '40), but in only one case was the father a twin (Gilmore).

[1] By personal communication with the attending physician, Dr. R. A. Gilmore, St. Anthony's Hospital, Michigan City, Indiana, January 27, 1941.

The age of the mother of the quadruplets described in this paper was 26 years. This figure is somewhat below the average as determined by Hauser ('13) and Brattström ('14), who point out that approximately 75% of the quadruplet mothers are 30 years of age or older. Figures obtained from the

TABLE 4

Family histories of multiple births. × = *twins.* + = *triplets.*

SOURCE	PATIENT	PATIENT'S MOTHER	PATIENT'S G. MOTHER	PATIENT'S G. G. MOTHER	PATIENT'S SISTER	PATIENT'S BROTHER	PATIENT'S AUNT	PATIENT'S COUSIN
Brattström		×	×		×			
Budd					×		×	××
Constantine		×	×			×		
Diddle and Burford	××							
Dawson		×	××	×				
Etheridge		××××						
Fathergill	×							
Fleming		×						
Frank	×		×				+	
Häfeli							×	
Miller	×		×				×	+
Papatestas			+		×			
Boston M. and S.[1]	×							
Roberts	×+×							
Rossi	×							
Sinclair		×						
Storz	×+							
Thompson								×
White					×		×	
Fotheringham [2]								
Willett [3]								

[1] Editor's note in Boston M. and S., 1922, vol. 142, p. 233.

[2] Maternal history but no relationship given.

[3] A niece has a multiple birth.

Department of Commerce, Bureau of Census, on 52 sets of quadruplets born in the years 1917–1939 and 29 other cases taken from the literature indicate the following frequencies for the various age groups: under 20 years, 5 cases; 20–24 years, 10 cases; 25–29 years, 13 cases; 30–34 years, 25 cases; 35–39 years, 26 cases; 40–44 years, 2 cases; 45 years and over,

1 case. Of the 81 cases considered 66.6% were over 30 years of age.

Hauser ('13) states that quadruplets are generally born to parents of six or more children. Rossi ('26) also concludes that multiple births increase proportionately with the number of children and that mothers of quadruplets are more prolific than mothers of twins or triplets. Brattström ('14) however, found in his study of 36 cases that 19 sets of quadruplets were born to mothers in their fourth or earlier pregnancy, while 17 were born in the fifth or later pregnancies.

From a study of 44 cases taken from the literature it was found that quadruplet births have occurred in all pregnancies from the first to twelfth. Twenty-four of the quadruplet births occurred in the fourth or earlier pregnancy, while twenty occurred in the fifth or later pregnancies.

SUMMARY AND CONCLUSIONS

A set of quadruplets, approximately 17 weeks old have been described, and upon the basis of a study of the fetal membranes it is concluded that four ova were involved in their production.

The family history of the mother reveals the occurrence of several previous multiple births.

A study of fifty-six cases of quadruplets indicates that quadruplet births are more frequent in families with maternal histories of multiple births than in those in which there are no multiple births.

The emphasis which has been placed by other authors upon the age and number of previous pregnancies of mothers of quadruplets has not been fully substantiated.

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A STUDY OF THE LIFE HISTORY OF CORTICO-ADRENAL GLAND CELLS OF THE RAT BY MEANS OF TRYPAN BLUE INJECTIONS

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ONE PLATE (FOUR FIGURES)

INTRODUCTION

Adrenal cortex cell types would appear from the evidence in the literature to be different stages in the life history of the same cell, and it is generally suggested that new cells arise in the glomerular region (Mulon, '03; Bogomolez, '09; Graham, '16; Goormaghtigh, '22; and Hoerr, '31), moving gradually toward the medulla. This conception is consonant with the finding that morphologic differences in adrenal glands with different functional loads are dependent on variations in the proportions of cell types usually present (Zwemer, '34, '36). A cytological study of the subcapsular region, the trabeculae and certain adenomata (Zwemer, Wotton and Norkus, '38) further suggested that typical gland cells might be derived chiefly from certain elongated capsular cells resembling fibroblasts. Evidence for the origin of adrenocortical cells from the capsule has also been presented by Baker and Baillif ('39) who enucleated the adrenal and subsequently used colchicine to arrest the mitoses of adrenal cells regenerating from the capsule. The fact that corticoadrenal gland cells may contain blue droplets after injection of trypan blue, has been demonstrated by Goldman ('09) and Hett ('27). An interpretation of its significance may have value in studies of the adrenal cortex.

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The present paper deals with an investigation of the possible origin of the glandular cells of the glomerular layer from the fibroblast-like cells in the capsule by marking the latter with trypan blue and sacrificing the animals at various periods thereafter. Using the same technique the movement of cortico-adrenal cells inward from the periphery was also studied.

METHOD

Subcutaneous injections of aqueous 0.5% or 1% trypan blue were given to thirty-seven rats from six litters of various ages and weights. The amount given daily and the responses of the animals can be ascertained from table 1. Groups of rats were injected for 2, 5 and more successive days and sacrificed at various intervals after stopping the injections.

The adrenal glands were fixed in Bouin's solution or neutral formalin. The most useful paraffin sections were those fixed in Bouin's fluid and mounted without staining, since the yellow color contrasted with the blue droplets and did not obscure them. Acid fuchsin was also used as a counterstain. Formalin-fixed glands were sectioned by the freezing technique, with and without gelatin embedding; then stained with sudan III or IV to determine whether fat droplets and trypan blue were in the same cell. These sections were mounted in "glychrogel" (Zwemer, '33; Wotton and Zwemer, '35). In studying the slides, classification into groups was done with no knowledge of the treatment to which the animals had been subjected.

OBSERVATIONS

It is well-known that after injection of trypan blue the dye is taken up first by macrophages. The accumulated dye appears in coarse blue droplets which may eventually occupy a large part of the cell. Later smaller droplets may be observed in the fibroblasts.

The adrenal capsule is rich in macrophages, but is composed mainly of fibroblast-like cells. Three days after the initial injection of trypan blue, the only cells in the adrenal beside the macrophages taking up blue dye were these fibroblast-like

cells in the capsule (table 1 and fig. 2). The remaining animals of this series received no further injections and were sacrificed 5 to 8 days later, and it was observed that some of the gland cells in the preglomerular and glomerular zones contained fine blue droplets. Since there were no injections after the first 2 days the blue-marked cells in the glomerular layer would

TABLE 1

Animals used, grouped according to treatment and distribution of cells containing blue granules in the adrenal cortex.

NUMBER OF RATS	DAILY DOSE OF TRYPAN BLUE	NUMBER OF DAYS INJECTED	DAYS BETWEEN LAST INJECTION AND AUTOPSY	TOTAL DAYS SINCE FIRST INJECTION	REGIONS WHERE CELLS WITH BLUE DROPLETS ARE MOST PROMINENT (MACROPHAGES EXCEPTED)
2	½–1 cc., 0.5%	2	1	3	Capsule only
4	½–1 cc., 0.5%	2	3–6	5–8 (ave. 6.5)	Capsule, preglomerular. Occasional glomerular cells
4	½ cc., 1%	5	1	6	Capsule, preglomerular. Occasional glomerular cells
14	½ cc., 1%	5	4–11	9–16 (ave. 12.2)	Some parts of capsule free of blue. Preglomerular and glomerular, blue. Occasional fascicular cells blue
4	½ cc., 1%	7	4–9	11–16 (ave. 13.5)	Some parts of capsule free of blue. Preglomerular and glomerular, blue. Occasional fascicular cells blue
4	½ cc., 1%	5	11–18	16–23 (ave. 20.7)	Capsule and preglomerular zones, little or no blue. Much in glomerular and outer fascicular cells
1	1½ cc., 1%	11	1	12	Preglomerular, glomerular and outer half of fascicular layer
4	1½ cc., 1%	14–20	1	15–31 (ave. 24.3) (only 1 rat less than 20 days)	Entire adrenal cortex including reticular layer

appear to be derived from the fibroblast-like cells in the capsule. In gelatin-frozen sections stained with sudan III or IV, some of the fibroblast-like cells were found to contain fine blue droplets and at the same time had a greater width than others, due to an accumulation of fine fat droplets. This is in accord with previous observations (Zwemer, Wotton and Norkus, '36), that lipoid-containing capsule cells were a transitional type.

Another series of animals were injected with trypan blue for a period of 5 days only, and killed at various intervals thereafter (table 1). On the sixth day after the initial injection, a few blue-containing cells were observed as far inward as the glomerular zone and occasional ones in the fascicular zone. The latter zone had some blue-stained cells after a period of 9–16 days (fig. 1) and many after 16 to 23 days (fig. 4), the extent of the blue in the fascicular layer being greater for the longer period.

In adrenals of animals killed more than a week after the last injection the capsule no longer contained as many blue cells as were seen in the first groups. A band of blue seemed to be moving inward, leaving a widening clear periphery. This clear area could not be demonstrated in animals which were subjected to longer periods of injection, 11–30 days (table 1). A wait of about 11 to 18 days after the last injection seems necessary to produce an outer clear band free of blue droplets, which is demonstrable without question (fig. 3).

It should be noted that the elapsed time after the first injection is an important element in determining the distance inward of the blue region. The rats sacrificed after about 20 to 30 days of injection showed that some of the blue cells of the cortex had migrated as far as the reticular layer (table 1).

DISCUSSION

The theory that the adrenocortical cells arise in the capsule does not, of course, deny their increase in numbers by mitoses in the glomerular and outer fascicular zones as has been observed by many investigators (see Hoerr, '31, for extensive

bibliography). The indifferent capsule cells which resemble fibroblasts took up trypan blue in the fine droplets. This type of droplet was found after a lapse of time in many of the glomerular and fascicular cells.

The typical gland cells of the glomerulosa and fasciculata which contained the blue granules contained also their fat inclusions, although the fat was reduced in amount and not as well distributed. Macrophages containing both trypan blue and fat were sometimes found among the glandular cells, but could be recognized easily by the size and type of their blue granules.

The rats did not exhibit toxic reactions after these doses of trypan blue; they gained weight and remained healthy. Since the rats were not under a severe strain which might affect the adrenals, the time required for blue-marked capsule cells of the adrenal to move inward might be regarded as approximating the normal. The schedule of the life cycle of an adrenal cell in the series of rats used would be about as follows: from capsule to glomerular zone—average 6 days; from capsule to beginning of fascicular region—average 12.2 days; from capsule to reticular layer—20 to 30 days. The range can be determined from the table, in which it may be observed that individual differences result in overlapping of groups.

A means of measuring such inward movement in animals with different conditions of adrenal strain would seem advantageous, since cells of the adrenal cortex appear to move from the periphery to the medullary border of the gland with a varying rapidity depending on the severity of the demand on the adrenal.

Animals can be given enough trypan blue to cause illness and loss of weight. This condition is usually attributed to a blocking of the reticulo-endothelial system. In animals having excessive trypan blue, not only the reticulo-endothelial system is loaded, but also a majority of the corticoadrenal gland cells may contain blue granules (see colored illustrations of Goldman, '09). The lipoid granules of cells containing many

trypan blue granules are less numerous and coarser than normal. Additional experimental work would be required to show whether symptoms usually attributed to blocking of the reticulo-endothelial system are in any way related to adrenal deficiency.

SUMMARY

Thirty-seven rats from six litters of various ages and weights were injected with relatively low doses of trypan blue. They were sacrificed at various intervals and their adrenal glands were sectioned and studied.

Animals injected for 2 days and killed on the third day showed (beside macrophages) small blue droplets only in the fibroblast-like cells of the capsule. On the sixth day (no additional injection), blue-stained cells were found in the glomerular zone. It is suggested that these glomerular cells were derived from the capsule.

Another group of rats were injected for 5 days and killed after varying intervals during which no injection was given. Six days after the first injection, blue-stained cells were found in the glomerular layer; after 12 days in the fascicular layer; and after prolonged injection, 20 to 30 days, in the reticular layer.

The fact that corticoadrenal cells move toward the medulla was further demonstrated in that after 5 days injection and a wait of 11 to 18 days, a band of blue-stained cells was found to be most prominent in the inner fascicular zone, leaving a clear band in the region near the capsule.

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PLATE 1

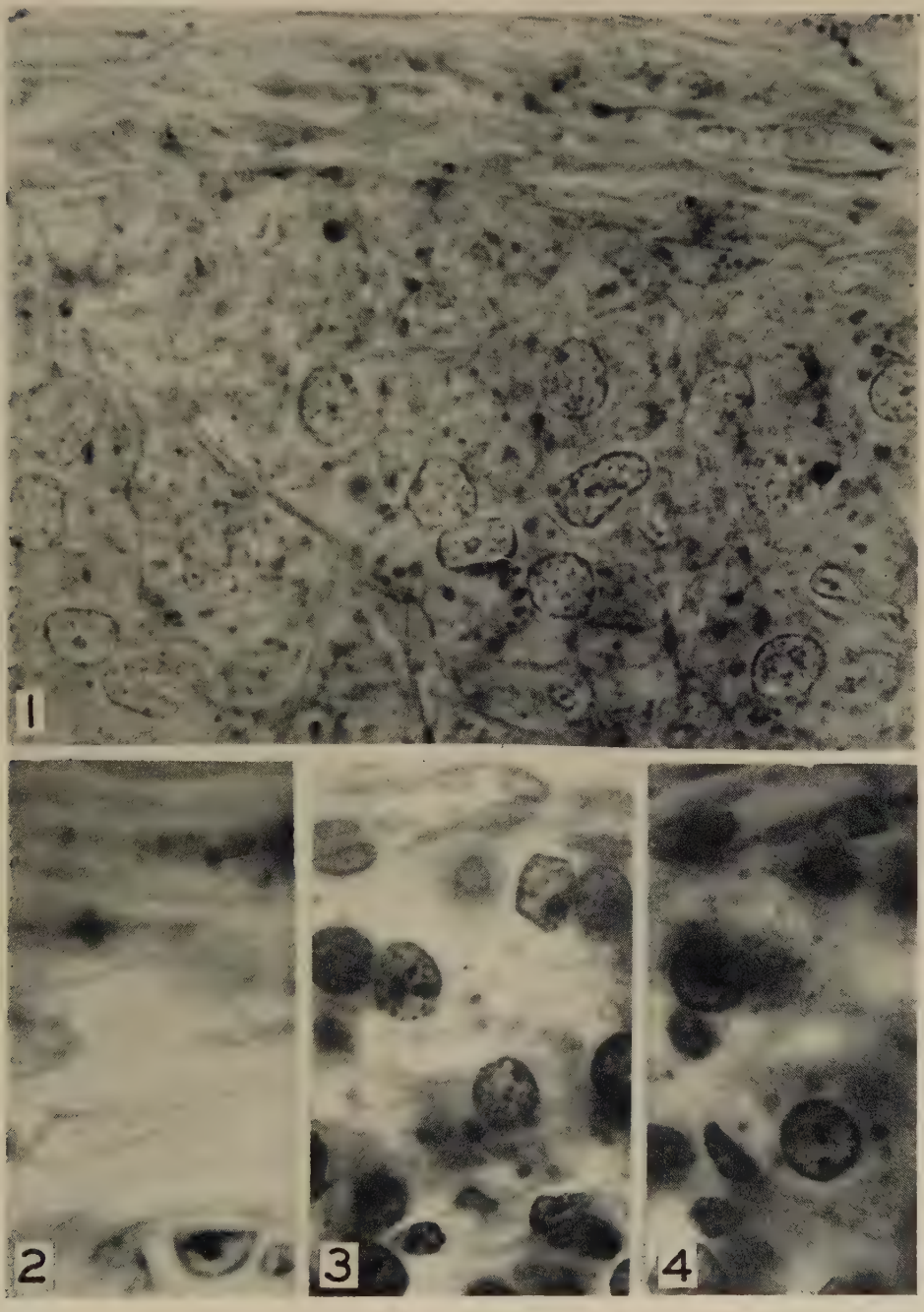
EXPLANATION OF FIGURES

1 Adrenal cortex from rat injected 5 days with trypan blue, killed 9 days after last injection. Dye droplets are seen in capsule, glomerular and also fascicular cells. (Low power.)

2 Trypan blue in capsule cells only. Rat was injected with trypan blue for 2 days and killed on the third day. (High power.)

3 Trypan blue in glomerular cells, but no longer in the capsule cells. Rat was injected with trypan blue for 5 days. Killed 12 days after last injection. (High power.)

4 Two typical outer-fascicular adrenal gland cells containing dye droplets. (Oil immersion.)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SURGICAL ANATOMY OF THE CAROTID SINUS NERVE

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TWO FIGURES

The anatomy of the carotid sinus and of its complex system of nerves has assumed increased importance since the surgeon has found denervation by periarterial sympathectomy of value in the treatment of carotid sinus syncope. Anatomical variations of this innervation are many, but from the surgical standpoint it is important to know whether there is a distinct carotid sinus nerve from the glossopharyngeal which could be recognized and divided at operation, or whether the nerves supplying the carotid sinus form what Hovelacque ('30) calls an intercarotid plexus, in which case an extensive periarterial stripping would be essential.

The discovery of the carotid body is credited to Haller who believed it to be a sympathetic ganglion. In 1833 Mayer described a plexus of nerve fibers in relation to it receiving branches from the glossopharyngeal, vagus and cervical sympathetic nerves, and Switzer in 1863 added the occasional contribution from the hypoglossal. The carotid sinus, as a slight dilatation of the internal carotid artery at its origin from the common carotid, had been described by Burns in 1811, but it was not until Hering's account of the mechanism of the carotid sinus reflex in 1924, and the physiological discoveries which followed, that attention became focused on the anatomical arrangement of its nerve supply. Accounts of the innervation, based on human dissections, have been given by Gerard and Billingsley ('23), Braeucker ('23), Drüner ('25), Danielopolu and Manescu ('28), Hovelacque

and his colleagues ('30), and by Tchibukmacher ('38). In most of these descriptions, however, the arrangement was that found in single examples, or where several dissections had been made, the number is not stated and it is not possible to draw any conclusion as to the relative frequency of variations likely to be found. Hovelacque's account is particularly interesting because it describes the formation by branches from the glossopharyngeal, vagus and cervical sympathetic, of a carotid plexus, fibers of which pass to both the carotid body and to the carotid sinus.

The variations in man in the innervation of this region have been recorded by Cordier and Coulouma ('32) and by Boyd ('37). The former, on the findings in thirty human dissections, described five types of communicating loops between the descending (carotid) branch of IX and neighboring nerves. In 93% of cases there was an anastomosis with the vagus, and in 16% (five dissections) there was an additional loop of communication with the sympathetic. Nevertheless the carotid branch of IX did not appear to be lost in a plexus, but could be traced clearly through to the sinus. Boyd's account ('37) of the nerve supply of the carotid sinus is more complete and is based on fifty-nine adult dissections, together with data on the arterial pattern from many other specimens. Concerning the carotid sinus proper, a dilatation of the internal carotid at its origin was present in 129 out of 143 carotids examined, and where it was not recognizable as such, there was usually a dilatation on the termination of the common carotid or on the commencement of the external carotid artery. The carotid sinus was more obvious in the aged than in young specimens, and the left sinus was usually better developed than the right. It is clear from Boyd's description that there is a distinct carotid branch from IX, but that it ends not only in the carotid sinus but also in the carotid body, in an intercarotid plexus or in both.

In this laboratory during the past 2 years special attention has been directed to this region in the course of routine student dissections, and in addition thirty-three special prep-

arations have been made, either by one of us or under our direct supervision. For help in these dissections we wish to thank many of our graduate surgical students. The observations recorded can only be regarded as supplementary to the more extensive investigations of Boyd ('37), and in view of the completeness of this latter work, the surgical standpoint has received the main emphasis.

There are three main nerves which contribute to the innervation of the carotid sinus, the glossopharyngeal, the vagus and the sympathetic. A branch from the hypoglossal is exceptionally rare and was not actually found in any of the special dissections made in this study. It soon became evident that branches of the three nerves mentioned above, reach the carotid sinus in two ways, directly along the so-called carotid sinus nerve proper, and indirectly through the carotid body. The former constitutes a distinct branch from the glossopharyngeal, arising close to its origin, and running down the anterior surface of the internal carotid artery. It forms loops of communication with branches of the vagus and cervical sympathetic as described by Cordier and Coulouma ('32), but its main pathway can in almost all cases be traced through directly to the carotid sinus. The second, more indirect route, appears to be equally distinct. An intercarotid "plexus" is formed, as described by Hovelacque ('30), principally by branches from the cervical sympathetic and vagus (particularly from the superior laryngeal nerve), and occasionally from the main trunk of the glossopharyngeal. This plexus lies posterior and medial to the internal carotid artery, and may communicate with the carotid sinus nerve proper or with its loop of communication with the cervical sympathetic. Many of the fibers of the plexus are destined for the carotid body, through which some appear to run to the carotid sinus. Both methods of innervation of the carotid sinus can be recognized in the majority of dissections, and a typical arrangement is illustrated in figure 1. The thirty-three special dissections have been utilized as the basis for the following account.

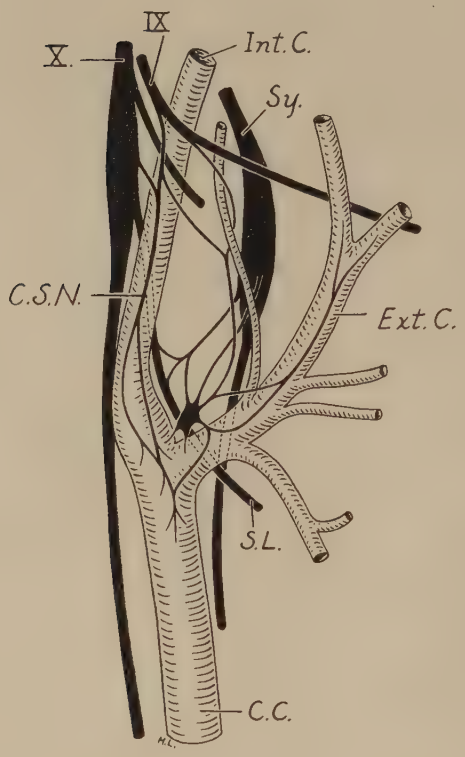


Fig. 1 Diagram of innervation of carotid sinus.

IX = glossopharyngeal nerve	S y = cervical sympathetic
X = vagus nerve	C. C. = common carotid artery
S. L. = superior laryngeal nerve	Int. C. = internal carotid artery
C. S. N. = carotid sinus nerve	Ext. C. = external carotid artery

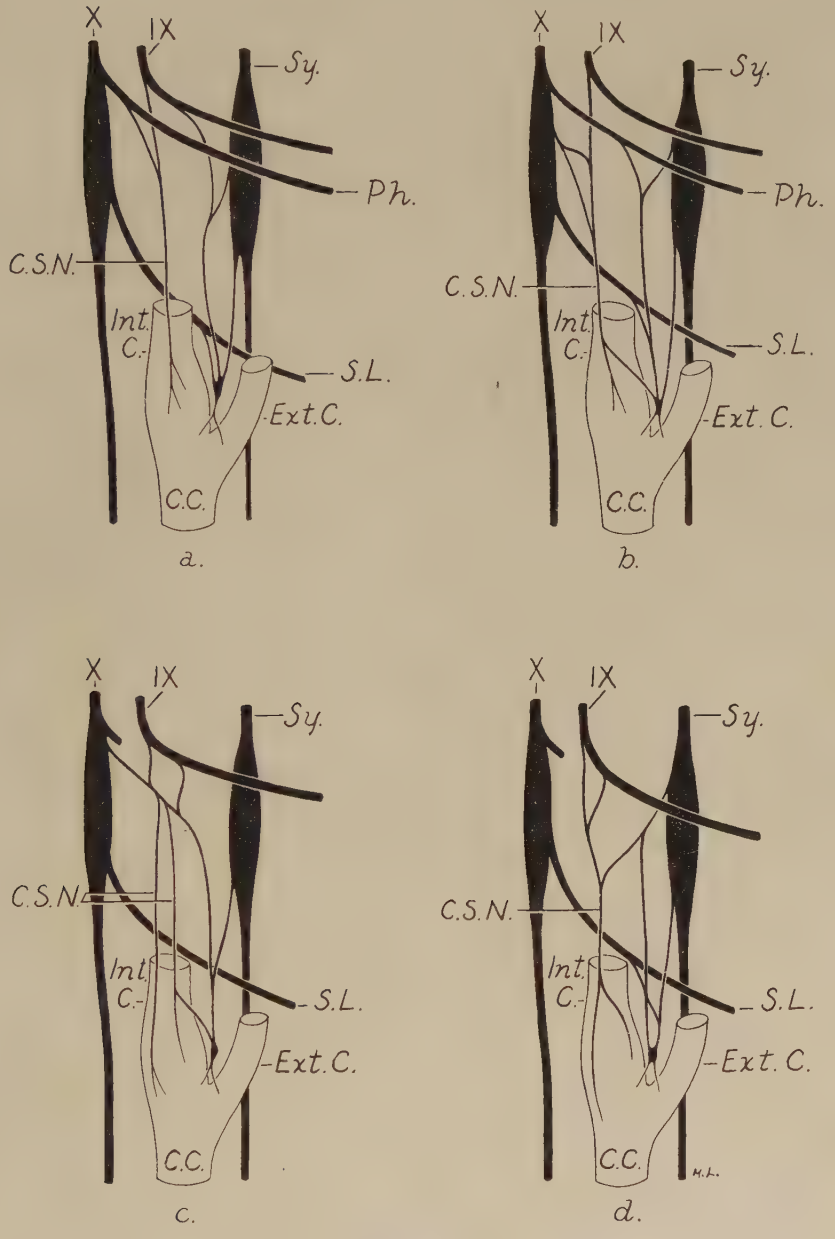
Fig. 2 Four common variations of innervation of carotid sinus.

(a) carotid sinus nerve from glossopharyngeal and pharyngeal branch of vagus.

(b) carotid sinus nerve from glossopharyngeal and nodose ganglion of vagus.

(c) carotid sinus nerve double.

(d) carotid sinus nerve from glossopharyngeal and cervical sympathetic.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C.S.N.
Int. C.
S.L.
Ext. C.
C.C.
b.
X
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Int. C.
S.L.
Ext.C.
C.C.
c.
X
IX
Sy.
C.S.N.
Int. C.
S.L.
Ext. C.
C.C.
M.L.
d.

Figure 2

Carotid sinus nerve proper

The anatomy was frequently different on the two sides of the same individual but there was no constant relationship. In all thirty-three preparations the carotid sinus nerve arose from the main trunk of the glossopharyngeal soon after its emergence from the jugular foramen, and in nineteen instances the point of origin was within 1 cm. of the base of the skull; in another twelve specimens the distance was 1–3 cm. and in only two cases was it as great as 4 cm. The high origin of the carotid sinus nerve is therefore a constant feature. The origin was double in over half the dissections, the two roots joining together within 1–3 cm. A complete double carotid sinus nerve (fig. 2 c) was only seen twice, although Boyd ('37) from a larger series has found this arrangement to be much commoner.

In its course along the internal carotid artery, which it followed, the nerve was directly anterior in eighteen instances, nine times antero-lateral and six times antero-medial. There is therefore no certain location for the nerve, but it should be sought directly on the sheath covering the anterior surface of the artery. Its length varied between 2 and 7 cm. (average 5 cm.) according to its point of origin and to the position of the bifurcation of the common carotid artery. The latter is an important surgical landmark. It is usually placed on a level with the upper border of the thyroid cartilage, but in ten of the present series of dissections it was found as high as the hyoid bone, and in only two cases it was below its usual position.

Loops of communication between the carotid sinus nerve and branches of the vagus and cervical sympathetic were very common, but the occasional contribution from the hypoglossal was not seen. The communication with the vagus proper (nodose ganglion), or with its pharyngeal branch (fig. 2 a, b and c) was by far the commonest (in twenty-six of the thirty-three dissections), and took the form of one or two loops on the lateral side of the artery. The com-

munication was placed high, soon after the origin of the carotid sinus nerve from the glossopharyngeal trunk; in no instance was any such communication found within 3 cm. of the carotid bifurcation, an important fact from the surgical standpoint. A connecting loop between the carotid sinus nerve and the superior cervical sympathetic ganglion was present in only nine instances, in seven of which the loop with the vagus was absent (fig. 2 d). The connection with the sympathetic was placed lower and against the medial aspect of the internal carotid artery, and formed a loop from which fibers also passed into the intercarotid plexus. Despite these communications, the carotid branch of the glossopharyngeal could be recognized as a distinct nerve passing down towards the carotid sinus. Only four times was the connection with the intercarotid plexus so complex that the identity of the carotid sinus nerve became lost.

One or more terminal branches of the carotid sinus nerve were almost invariably traced to the bulbous origin of the internal carotid artery where they appeared to end, and in twenty-two cases another branch passed medially to enter the upper pole of the carotid body, sometimes connecting with the intercarotid plexus at this point. From the lower pole of the carotid body fine nerve twigs branched out to the carotid bifurcation and so presumably to the carotid sinus.

Intercarotid plexus

An intercarotid plexus of varying complexity was clearly recognizable in all cases. The vagal contributions came most often (twenty-three examples) from the superior laryngeal nerve as it passed behind the carotid artery, but other branches from the nodose ganglion (eight examples) and from its pharyngeal branch (five examples) were also traced (fig. 2). The superior cervical ganglion always sent one to three small nerves which looped with the vagal branches to form the plexus lying between the internal and external carotid arteries. The contribution from IX took the form either of a direct branch from the glossopharyngeal trunk (fig. 2 a) or

of a loop between the sympathetic and carotid sinus nerve (fig. 2 d). In many of the dissections the only branch from IX was one of the terminal branches of the carotid sinus nerve entering the intercarotid plexus at the upper pole of the carotid body (fig. 2 b and c).

The distribution of the fibers of the plexus was difficult to determine. Some of the branches invariably passed to the lateral pharyngeal wall where they became lost in the pharyngeal plexus. Others were distributed to the external carotid artery and its branches, some being traced towards the thyroid along the superior thyroid artery. Others passed directly downwards to the carotid bifurcation and entered the upper pole of the carotid body. This body, though varying greatly in size and colour, was recognizable in almost all dissections, as a minute fleshy knot within the bifurcation. From its lower pole fine twigs radiated to the internal and external carotid arteries, and so to the sinus wall.

DISCUSSION

The anatomical variations as described here confirm many of the features described by previous investigators, and do not therefore require further comment. We wish to draw particular attention, however, to the constancy of a distinct carotid sinus nerve from the glossopharyngeal and its relative separation from the intercarotid plexus. It is true that there is a communication between the carotid sinus nerve and the plexus, but the former can still be traced along the anterior wall of the artery to the carotid bifurcation. The anatomy suggests that, if such a procedure should prove advisable, it would be possible to section this nerve alone and avoid an extensive stripping of the internal carotid. Section of the carotid sinus nerve just above the bifurcation would of course leave intact fibers reaching the sinus along the second route, namely through the intercarotid plexus and carotid body, but the operation has been tried in certain cases because isolation and division of the nerve alone was followed in these patients by an immediate rise in blood pressure. The

hypertension is not persistent (Capps and de Takáts, '38) for the aortic depressor and other mechanisms are intact. Though the relief from attacks in patients with hypersensitive carotid sinuses, following this or any other form of carotid sinus denervation, is encouraging, a note of warning must be sounded, for regeneration within the peripheral autonomic nervous system is extraordinarily rapid and difficult to prevent.

Despite Drüner's observation, we have chosen to retain Hering's term carotid sinus nerve because of its physiological implications, rather than revert to a purely morphological designation. True this gives undue emphasis to the innervation of the sinus and ignores that to the carotid body, but until the functional difference between these two structures has been clarified, terminology may logically follow in the footsteps of physiology which at present endeavours to interpret a complex system of carotid sinus reflexes in the regulation both of circulation and respiration. The histology of the carotid sinus, the thickening of the adventitia, the thinning of the media and the specialized disc-like sensory receptors in its wall, have been fully described by de Castro ('26, '28, '29), Riegele ('28) and by Sunder-Plassmann ('30). Boyd ('37) has shown how changes in blood pressure are mechanically magnified at any dilatation, and that the thinning of the media allows impulses to be transmitted more readily to the nerve endings in the thickened adventitia. Hence the anatomical arrangement has functional significance.

The physiological nature of the carotid body is not clear. It has been assumed at different times to be a sympathetic paraganglion containing chromaffin tissue, an endocrine gland and a sensory receptive organ responsive to chemical changes in the blood. Hence the various names ganglion, gland, globus, etc., which have been used at different times for this structure. Nerve fibers form a dense network both around and within its substance, and although nerve terminals have been seen in the neighborhood of the cells, no true neurones have been described. Hence it is difficult to regard the carotid

body as an autonomic ganglion, or as belonging to the paraganglionic system, for chromaffin tissue is present only in certain animals, and then almost always on the surface. Ask-Upmark ('35) in summarizing the functional relationship between the carotid sinus and the carotid body states that the sinus is unquestionably receptive to hydrodynamic stimuli, consequent upon changes in blood pressure. The carotid body on the other hand is probably sensitive to certain chemical alterations, such as anoxemia, and in addition may or may not act as an endocrine organ, the only positive evidence in this regard being the cytological structure, and the possibility of extracting from it, as demonstrated by Christie ('33), a substance antagonistic to adrenalin.

The glossopharyngeal branches have generally been considered as transmitting the impulses from the sinus wall, and possibly from the carotid body, although some authors have stressed the importance of the sympathetic and of the vagus. Embryologically (Kohn, '00; Rabl, '22; Smith, '24; Hammer, '34 and Boyd, '37) the mesodermal condensation related to the artery of the third branchial arch is also directly concerned with the development of the carotid body. The glossopharyngeal is of course the nerve of this arch, but more important is the fact that its contribution to the developing carotid sinus and carotid body is present in all human embryos from the 8 mm. stage, whereas it is not until the 26–30 mm. stage that cells can be traced to the carotid body from the vagal trunk, and not until the 30–36 mm. stage that the contribution from the cervical sympathetic becomes evident. The importance of the ninth nerve contribution is also supported by other lines of evidence, by its striking constancy in all species of animal (Ask-Upmark, '35) and its singular limitation to the carotid artery, whereas the vagus and sympathetic send branches along blood vessels elsewhere in the body.

The sympathetic contribution is composed almost entirely of unmyelinated fibers and probably, like the sympathetic innervation elsewhere, is vasomotor in function. The vagal

contribution, however, cannot be lightly disregarded, for it contains many large myelinated fibers, apparently afferent in nature. The physiological evidence on this point is not conclusive, and on account of the frequent communication between the vagus and the carotid sinus nerve, surgical division of the latter unquestionably includes vagal fibers. Morphologically, however, the afferents of the ninth and tenth cranial nerves belong to the same category, so that the distinction may be more of practical than of fundamental importance.

SUMMARY

The common variations of the carotid sinus nerve and of the innervation of the carotid body have been described, based on thirty-three special dissection of this region.

The carotid sinus is innervated by the glossopharyngeal, vagus and cervical sympathetic, and exceptionally by the hypoglossal. The fibers reach the sinus in two ways, (1) along the carotid sinus nerve, and (2) through an intercarotid plexus and the carotid body.

The anatomy suggests the feasibility of dividing the carotid sinus nerve in preference to extensive stripping of the carotid artery, in the surgical treatment of carotid sinus syncope.

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THE NUMBER OF EGGS AND SURVIVING EMBRYOS IN ELEPHANTULUS

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THREE FIGURES

INTRODUCTION

The uterus of the elephant shrew, Elephantulus myurus jamesoni, is Y-shaped, consisting of a median part and two long horns. The gravid female invariably has two embryos, one in each horn, lodged a little distance above the junction of the horns with the median uterus (fig. 1).

Elephantulus myurus jamesoni is quite a common animal on the High Veld of the Transvaal. During the last few years we were able to make an extensive collection of embryological material of this little animal. Although several hundreds of gravid uteri were examined, no exception was found to the rule that this animal has a single embryo in each uterine horn.

According to Shortridge ('34) two embryos are also the rule in other species of Elephantulus and Macroscelides. In Nasilio, on the other hand, Shortridge found that of five pregnant females three had single embryos and two had twins, while in Petrodromus tetradactylus there were single embryos present in all three females examined. However, it is beyond dispute that Elephantulus myurus jamesoni always carries twins.

In sharp contrast with this small and constant number of embryos that reach maturity is the enormous number of eggs set free from the ovaries at each ovulation. The greatest number of eggs counted from one ovary was sixty-six; this

number was found in two different animals; in others fifty-three, forty-seven and fifty-seven have been observed. This means that at each ovulation about 120 eggs are liberated from the two ovaries. It is hardly necessary to say that the two ovaries ovulate simultaneously.

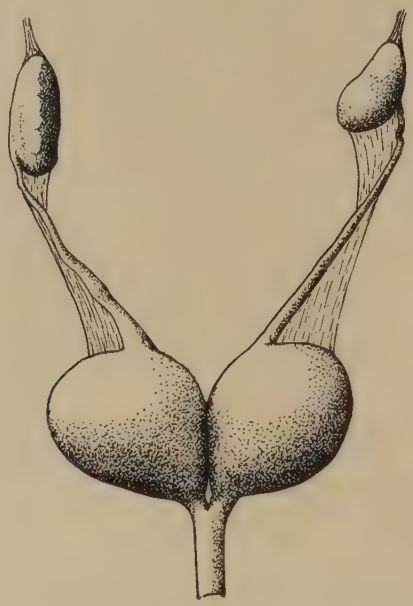

Fig. 1 The genital tract of a gravid female. × 3.

THE NUMBER OF EGGS

This enormous number of eggs, 120 liberated at each ovulation, is far greater than that recorded for any other mammal, even the most prolific species. Hartman ('25) found that in the opossum the average number of eggs discharged at one ovulation is twenty-two (eleven from each ovary); although he states that once he found forty-three and even fifty-six, but the liberation of more than thirty eggs at a time is rare. According to McCrady ('38) as many as twenty-two ova from each ovary have been observed in the opossum. In Hemicentetes Bluntschli ('37) found up to twenty early developmental stages in each uterine horn, even though the

maximum size of the litter is only ten. In Centetes the greatest number of older embryos, found by the same author ('38) was thirty-two and forty in the earlier blastocyst stage, but in this case the number of liberated eggs is not stated. In both the opossum and Hemicentetes the number of eggs is far less than 120, the number that may be found in Elephantulus, and furthermore the size of the litter of these animals is neither small nor fixed as it is in Elephantulus. In other mammals, as far as is known, the number of eggs discharged at one ovulation is less than in the opossum or Hemicentetes, though it mostly exceeds the number of embryos that reach maturity.

The number of discharged eggs can be considerably increased artificially in mammals. Smith and Engle ('28) have shown that in mice and rats, injected with fresh anterior pituitary, as many as forty-eight eggs were liberated into one Fallopian tube; the smallest number in their experiments was twenty-two for the two tubes, fourteen in the right and eight in the left, and even this exceeds very considerably the greatest normal number ever reported for these rodents. Pincus ('40) recovered up to eighty-two eggs from rabbits injected with pituitary extracts. In Elephantulus, however, no injection is necessary; the great number of liberated eggs is here a normal feature. But considering the results of the above mentioned experiments one might conclude that in Elephantulus either the pituitary secretes potent gonadotropic hormones or else that the ovary responds in a prolific fashion to ordinary physiological stimuli.

DISTRIBUTION OF THE EGGS

After their discharge from the ovary the eggs of Elephantulus travel quickly through the periovarial space into the ampullary part of the Fallopian tube. That this relatively long distance is covered in a short time is clear from the following considerations. In a previous study (van der Horst and Gillman, '40) we showed that the eggs of the sixty odd Graafian follicles in each ovary are not liberated simulta-

neously; all stages from ripening follicles to young corpora lutea are found in one and the same ovary. It therefore takes without doubt a considerable time, maybe a few hours, before all the eggs are discharged at each ovulation. In addition since the ostium tubae is located near the posterior end of the elongated ovary, there is a marked discrepancy in the distance to be covered by individual eggs from their place of discharge in the ovary to the entrance of the Fallopian tube. Despite this a procession of eggs over the surface of the ovary was found in only a few of our many series of sections.

Having covered this distance, the eggs are all gathered in a special dilated part of the Fallopian tube, such as has been described by Sobotta ('16) in various rodents. It is in this chamber that the eggs stay for a considerable time, that the second polar bodies are formed, that the eggs are fertilized and that the first segmentation may take place.

In several of our series of sections we found the eggs massed together in this dilated part of the Fallopian tube where their number can be counted (fig. 2). Counting eggs even in this region is a laborious task. When a great many of eggs are massed together and often pressed against each other, it becomes difficult to identify unsegmented eggs from two-cell stages which may be found intermingled in this region. One has to outline and measure each egg in every section in order to make sure that every egg is counted once only.

Young four-cell stages are occasionally found in the dilated part of the tube also, but mostly the eggs seem to pass through the pars isthmica and to enter the uterus before they reach this stage. On the other hand, undivided eggs and two-cell stages that seem to be perfectly healthy may be found in the upper regions of the uterus. Here one can sometimes see all stages from the undivided egg to the old four-cell stage mixed together, and this mixing is at random so that the unsegmented eggs are not higher up in the uterus and the older stages lower down. The egg reaches the implantation site always in the old four-cell stage and the early

embryos, lying free in the uterus, never go beyond that stage in their development.

Although we have at our disposal several series of ovaries at a period shortly after ovulation, we counted the exact number of eggs in the five instances mentioned above only.

Fig. 2 Section of the Fallopian tube containing a great number of eggs. × 100.

These five cases are sufficient to allow us to state that at each ovulation an average of sixty eggs is discharged from each ovary.

FACTORS RESPONSIBLE FOR THE REDUCTION IN THE NUMBER OF EGGS

There is one remarkable feature which needs closer examination. As has been emphasized above a large number of

eggs are discharged from the ovary in Elephantulus during ovulation, many of which develop to the four-cell stage, and yet only one embryo continues to develop successfully in each uterine horn. A mechanism must be present, therefore, for limiting in a drastic fashion the number of eggs discharged. There are a number of factors which either acting alone or in conjunction with each other seem to play a part in this reduction of the number of eggs.

Firstly a number of eggs, mixed with perfectly healthy ones, apparently degenerate before or even after they are set free from the ovary. These degenerated eggs are very vacuolated. When such eggs are just discharged from the ovary the nucleus still seems to be normal, but soon it also shows obvious signs of degeneration. The vacuoles become larger but fewer in number, so that the cytoplasm is more and more reduced to thin shreds compressed between the huge vacuoles. At the same time the cell shrinks and becomes irregular in shape. Occasionally one can find these older vacuolated eggs. Ultimately they lose their contents and all that remains is a shrivelled up cell membrane; often such cell membranes are found lower down in the uterus. It has not been possible to establish whether these degenerated eggs are ever fertilized; at any rate they were never found in the two-cell stage. This same form of degeneration also occurs in great numbers of eggs in all stages of development while they are still in the ovary. Apparently the vacuolar degeneration in the discharged eggs sets in only at the very last moment preceding the rupture of the Graafian follicle. Even though these degenerated eggs are not numerous, they account at least for the elimination of some of the eggs discharged from the ovary.

Secondly not all the eggs may be fertilized. That this is actually the case is shown by the fact that amongst many eggs that are fertilized one can often find a greater or smaller number that show the characteristics of unfertilized eggs. These eggs undergo changes similar to those described by Margaret Mann ('24) in unfertilized tubal eggs of rats. The

ripening spindles may lie parallel to the surface of the egg. Eggs are sometimes found containing a number of small nuclei, each nucleus being formed by one or a few chromosomes with some fluid round them, and these small nuclei are not of uniform size. Charlton ('17) also found that in unfertilized eggs of the mouse the second maturation spindle usually breaks down into many nuclei. These multinucleated eggs occur even in the Fallopian tube of Elephantulus, whereas Smith ('25) found that in the opossum only the uterine eggs show the earliest stages of degeneration. In the upper part of the uterus of Elephantulus degenerating eggs were also found in great numbers, especially in uteri in which the one successful egg had reached its place of implantation lower down in the uterine horn. These unfertilized eggs in the upper part of the uterus are quite dull; only rarely in the earlier stages can a faint indication of a nucleus be seen. Ovulation without insemination is a regular phenomenon in Elephantulus and then, of course, all ova show the characteristics of unfertilized eggs as described above.

Thirdly prenatal death, which is a common phenomenon in mammals, might be considered. According to Allen, Kountz and Francis ('24) the prenatal mortality in pigs is 30 to 46% and Droogleever Fortuyn ('29) found 38.34% empty egg-chambers in the striped hamster. However, it is clear that prenatal death as applying to other mammals is not responsible for this reduction of the number of eggs in Elephantulus. This is indicated by the fact that in Elephantulus the reduction in the number of eggs from sixty to one is achieved at the latest in the four-cell stage and before implantation, whereas prenatal death may occur at any stage of development.

These three factors, the presence of degenerate eggs, of unfertilized eggs and prenatal death, and perhaps others of the same nature of which we are unaware, certainly play a part in eliminating eggs, but they all act indiscriminately; they can account for a certain reduction in the number of

developing eggs, but they do not explain that always only one egg will continue its development in each uterine horn.

From the structure of the oviduct one might be tempted to think that the pars isthmica or the complicated tubo-uterine junction might play a role in the reduction of the number of ova even to such an extent that only one egg successfully might pass these parts of the genital tract. The pars isthmica

Fig. 3 Section through the pars isthmica of the Fallopian tube with three eggs. × 150.

is so narrow that the eggs can move through it only successively and therefore it would be possible that only the first egg to pass would reach its destination (fig. 3). However, in some instances it has been noticed that this first egg was clearly degenerate. The tubo-uterine junction could possibly act as a kind of trap, but we have never found eggs trapped in this region. That these parts of the duct can not act in some mechanical way in the elimination of eggs is proved unequivocally by the fact that a number of healthy and de-

veloping eggs may occur in the uterine horn. In one horn nine four-cell stages were found and some more in the tube, besides a number of two-cell stages and single eggcells, all of them looking quite healthy; it was impossible to predict which of them would survive.

So all these factors, enumerated above, can not account for the fact that only one egg from the sixty discharged from each ovary at ovulation will develop successfully. The deciding factor in this respect is to be found in the remarkable structure of the uterus. As we will demonstrate in another communication concerning the menstrual cycle in Elephantulus, there is only a very small and well circumscribed area in each uterine horn which will allow the successful implantation of a single embryo at a time. Other embryos may attempt to survive by exciting the formation of primary eggchambers, i.e., round diverticula of the uterine lumen. Very frequently embryos of four cells have been seen to occupy these primary eggchambers, but they never develop beyond the four-cell stage. It is only at a small and well circumscribed area of the uterus that the reaction of the endometrium goes further than the mere formation of an eggchamber; when an egg reaches this particular spot implantation takes place. The presence of this small area, where implantation can take place, is the only factor directly responsible for eliminating all but one of the sixty odd eggs discharged at ovulation.

SUMMARY

During ovulation Elephantulus myurus jamesoni can discharge on an average of sixty eggs from each ovary. This is by far the largest number of eggs liberated at ovulation by any mammal. On the other hand, the number of developing embryos is absolutely constant in this animal; one embryo develops in each horn of the uterus. The elimination of the excess of fertilized eggs is determined almost exclusively by the fact that there is only a very restricted area in each uterine horn which will allow the successful implantation of only one embryo.

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THE METHACRYLATE PLASTICS AS MOUNTING MEDIA FOR BIOLOGICAL MATERIALS

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ONE PLATE (SIX FIGURES)

INTRODUCTION

In an earlier note, Puckett ('40), the use of one of the clear plastics, ethyl methacrylate, as a mounting medium for embryological specimens was briefly described. Since the publication of this note a thorough investigation of several of the clear methacrylate plastics as mounting media for various types of biological materials has been made. This investigation has shown that these clear plastics are ideal for mounting various biological materials and this paper proposes to describe the technics employed in the use of several of these clear plastics.

1. MATERIALS AND METHODS

Two of the clear methacrylate plastics, ethyl methacrylate and methyl methacrylate (Plexiglas of the Rohm and Haas Co.; Lucite of the Dupont Co.), have been used in this investigation. Since the procedure employed in the use of each of these materials is identical, the use of only a single one, ethyl methacrylate, will be described. The properties of the hardened polymers of these two plastics are very similar, with the exception that methyl methacrylate is a bit harder and less liable to scratching. The writer recommends that persons using this technic for the first time use ethyl methacrylate as first results are liable to be better with this material.

A. Source of material. The unpolymerized ethyl and methyl methacrylate monomers used in this investigation were ob-

tained from the Rohm and Haas Chemical Co., West Washington Square, Philadelphia, Pa. The monomers come as clear liquids and are best obtained in 1 gallon lots.

B. Removal of inhibitor. The unpolymerized monomers are shipped with an inhibitor, hydroquinone, which is added to prevent a spontaneous polymerization. The inhibitor must be removed before polymerization. The inhibitor is removed by washing a sample of the monomer (500 cc. samples are used by the writer) four or five times with a 5% KOH solution. Washing should be repeated until the KOH solution ceases to be discolored by the hydroquinone. The sample is then washed several times with tap water to remove all traces of the KOH solution. Dehydration of this inhibitor-free monomer is accomplished by allowing the sample to stand over anhydrous sodium sulphate for 12 hours or longer. When filtered the material is ready for polymerization.

C. Preliminary polymerization of the monomer. Polymerization or hardening of the liquid monomer is effected by a catalyst, benzoyl peroxide. A stock solution of the catalyst is made by dissolving 5 gm. of benzoyl peroxide in 100 cc. of the inhibitor-free monomer prepared in section 1-B. This solution should be stored in a cool place. When the sample is ready for polymerization, this solution of the catalyst should be added in the ratio of 1 part to 10 parts of the inhibitor-free monomer. The sample is placed in a large, well corked Erlenmeyer flask for polymerization. This flask is placed in a water bath at 85°C. The flask should be continually agitated while in the water bath. Every 2 or 3 minutes the flask should be removed from the water bath, the cork removed to admit air, replaced and the flask well shaken. This treatment allows for a dissipation of the heat generated in the polymerization reaction. If this procedure is not carefully followed, the reaction will get out of control and a rapid boiling and hardening of the material will result in a loss of the entire sample. After 20 to 30 minutes of heating and shaking the sample will become partially polymerized and will have a viscosity about like that of molasses. At this point

the flask should be well cooled, corked to keep out moisture and placed in a refrigerator until needed.

D. Final polymerization of the monomer. Small, thin-walled glass preparation dishes make very good molds for the final hardening of these plastics. A dish of this type, holding about 25 cc., is filled to a depth of ¼ inch with the partially polymerized plastic prepared as in section 1-C. The dish is well covered and placed in an oven at 50°C. where polymerization is completed. This last step usually requires 24 to 36 hours and results in a very hard clear material similar to the clear plastics known commercially as Plexiglas and Lucite. A disk made as just directed should be kept in the glass dish to serve as a base for mounting some object by the technics which will be described in later sections.

E. Sawing, grinding and polishing hardened plastics. The polymers of both ethyl and methyl methacrylate are sufficiently hard to withstand sawing, sanding or cutting with a lathe. Sawing is best done with a thin hack-saw. After sawing, the original smoothness and polish of the plastic can be restored by grinding the cut surface with a series of fine sand papers down through no. 3-0 paper. This is followed by fine emery paper and the original polish is restored by a very fine abrasive such as Gorham's silver polish or rouge.

2. THE TECHNIC OF MOUNTING BIOLOGICAL MATERIALS IN THE METHACRYLATE PLASTICS

In the preceding section of this paper the technic for hardening or polymerizing the methacrylate plastics has been described. In the following section it is proposed to describe technics by which various types of biological materials can be embedded in these clear plastics.

A. Mounting embryos or other small objects (figs. 1 to 4, inclusive). Various types of biological materials, too large to be mounted under balsam on glass slides, or still larger objects, which normally must be displayed in dishes of some preservative, are easily embedded in the methacrylate plastics. As an example, the technic employed in mounting the 3-day

chick embryo shown in figure 1 will be described. The specimen is prepared in the same manner as for balsam mounting or for sectioning. The fixed embryo is stained with one of the common "in toto" stains as borax-carmine, haematoxylin or safronylin, dehydrated in a series of alcohols and cleared in xylene. From xylene it is placed in a dish of the inhibitor-free monomer (see section 1-B) for 24 hours to displace the xylene. The embryo is now placed in a glass preparation dish containing a disk of the hardened plastic which will serve as a base for the finished cast. The preparation is well covered with the thickened partially polymerized monomer, the dish covered and placed in an oven at 50°C. until well hardened. By mounting the embryo on a previously hardened disk of the plastic, it lies in the center of the finished cast. When completely hardened, the specimen is chilled with ice water, which loosens the plastic from the glass dish. No satisfactory way of getting the cast out of the glass dish has been found and, in most cases, the dish must be broken.

In addition to chick embryos of various ages which have been mounted in this way, various other objects have been successfully mounted. These include mammalian embryos (fig. 2), Amblystoma larvae (fig. 3) and a variety of other objects, including small fish, delicate skeletal parts, small plants and insects.

Dried insects are easily mounted. Figure 4 shows a large butterfly mounted in a block of this plastic. The dried specimen need not be dehydrated but is merely covered with the inhibitor-free monomer for 24 hours and then embedded in exactly the same manner as the chick embryo just described.

B. Mounting amphibian eggs and embryos (fig. 5). One of the problems confronted by the teacher of embryology is a suitable method of mounting amphibian eggs and embryos for student examination. The usual method of studying these objects in dishes of preservative presents objections such as the liability to breakage by the students; the difficulty in orienting the eggs properly, as well as the time required by the instructor in preparing this material each year. The clear

plastics appear to offer a solution to these problems. The following section describes a method for embedding frog eggs in disks of the clear methacrylate plastic. A preparation made in this way is shown in figure 5.

Eggs of the frog in various stages of development from the uncleaved ovum to the young tadpole stage, which have previously been fixed and washed are placed in dilute solutions of Eau de Javelle (sodium hypochlorite solution) until the membranes are completely digested away. The eggs are then well washed in tap water, dehydrated in a series of alcohols, but clearing in xylene is not necessary. The next step is to place the eggs in a dish of the inhibitor-free monomer for several hours. From the monomer they are transferred to a preparation dish containing a previously hardened disk of the plastic and properly oriented on this base. The partially thickened monomer (section 1-C) is then added to a depth sufficient to well cover the eggs, the dish covered and returned to the oven at 50°C. until well hardened. The final product is the disk shown in figure 5, which contains all of the early stages in the development of the frog and which is easily studied under the low magnification of a hand lens or dissecting microscope.

Sections through eggs and embryos embedded by this technic are easily obtained. A disk containing several eggs of the desired stage of development is ground on a sanding machine until the desired level in the eggs is reached. The original smoothness and polish of the ground surface is replaced with the use of fine sand paper and silver polish. Hemi-sections of frog blastulae and gastrulae prepared in this way make valuable demonstration preparations. If the eggs have been well impregnated with the plastic, grinding causes no breakage or distortion of the cells and the section obtained shows faithful details.

C. Mounting objects on plastic slides (fig. 6). If it is desirable to mount small objects such as chick embryos on unbreakable plastic slides, rather than in small plastic disks as described in section 2-A, the following technic should be used.

Polished sheet Plexiglas (methyl methacrylate), 0.06 inches in thickness and cut to standard microscope slide size, 3×1 inches, can be purchased from the Rohm and Haas Co., Philadelphia. Rings for making the cells in which the embryo is to be mounted are made in the following way. Polished Plexiglas rod, $\frac{3}{4}$ inch in diameter can be obtained from the Rohm and Haas Co. Using a lathe, this rod is converted into tubing with a bore of $\frac{5}{8}$ inch. With the cutting tool of the lathe, this tube is cut into rings of the desired thickness. The rings used in mounting chick embryos should range in thickness from 2 to 5 mm. These rings are fastened to the Plexiglas slide with a cement, Acryloid B-7 (Rohm and Haas Co.), which is a 20% solution of the polymerized methyl methacrylate in ethylene dichloride. The embryo to be mounted is prepared in the same way as for mounting in a disk (section 2-A). The embryo is transferred from the dish of inhibitor-free monomer to the cell just prepared, well covered with the thickened monomer, the cell covered with a cover glass and the preparation placed in an oven at 50°C. until well hardened. When well hardened, the cover glass should be removed and the preparation ground down almost to the level of the embryo in order to make as thin a preparation as possible. After grinding, the surface is polished as described in section 1-E. A 3-day chick embryo mounted in this way is shown in figure 6.

This technic is very valuable in mounting large chick embryos, 3 to 5 days of incubation, which are too large to mount under balsam or clarite on glass slides. These preparations take years to harden and are easily broken if touched by the objectives of the microscope. These plastic preparations are unbreakable and will stand a great amount of abuse by students.

D. The methacrylate plastics as embedding media in making sections of bone and other hard objects. The technic to be described appears to have wide applications in making sections of calcified bone, teeth and other hard objects which cannot be sectioned by ordinary methods. This technic will be described as applied to dried bone.

A piece of thoroughly dried bone such as a section of a cat femur or humerus, measuring about 1 inch in length is placed in a dish of the inhibitor-free ethyl methacrylate monomer for 48 hours to insure a thorough impregnation of the plastic. The bone is then placed in a glass mold, well covered with the partially polymerized monomer and the whole hardened as described in section 1-D. When well hardened the cast is removed from the glass mold for sectioning. The plastic cast is firmly attached in a vise and sections of bone about 1 mm. in thickness are cut with a fine hack-saw. A good number of these wafers, each containing a section of bone can be cut from the cast made above. One surface of a single one of these wafers is ground and polished as described in section 1-E. The polished surface of this wafer is fixed to a Plexiglas slide, using the cement, Acryloid B-7. When the cement is well hardened, the free surface of the wafer is ground to a thickness of approximately 10 to 15 μ and then well polished. During the grinding and polishing operations, it is well to bind the plastic slide in tin or copper foil in order to prevent a scratching of the slide. The finished section may be left uncovered or can be mounted in balsam or Clarite under a plastic or glass cover slide. Sections made in this way give a beautiful picture of bone structure and thinner sections can be made than when using the older technics of grinding without the bone embedded in a solid matrix. Sections of fresh bone and teeth which have been fixed, stained and dehydrated can also be made with this technic.

DISCUSSION AND SUMMARY

In the preceding pages the use of several of the methacrylate plastics as mounting and embedding media for certain types of biological materials has been described. By way of summary, certain of the advantages of this method will be discussed.

1. The cost of the material is relatively small and the technic for polymerizing the monomers and embedding ma-

terials a simple one. With little practice any worker should be able to obtain bubble-free casts.

2. The method is an ideal one for mounting older chick embryos, small mammalian embryos and similar small objects for study by students of embryology. Objects of this type are too large for mounting on glass slides and usually have to be studied in dishes of preservative. Mounted in a clear plastic, they are easily studied under low magnifications and with a minimum of trouble to the instructor as the preparations are relatively indestructible.

3. Life histories and developmental stages of certain common laboratory forms such as the frog can be mounted in a single disk for class study. These perparations are easily studied under a dissecting microscope and are far superior to the present methods of presenting this material for student study.

4. Preparations consisting of small embryos embedded in a clear plastic and mounted on plastic slides possess certain advantages. These preparations are unbreakable and this one characteristic makes these preparations worth-while as the breakage of whole mounts by beginning students is an item to be considered in any laboratory. The optical properties of these plastic preparations are fully as good as those of balsam-glass preparations when studied under the dissecting binoculars or with the lower objectives of the compound microscope.

5. These plastics appear to have wide applications in making sections of objects which, due to size, hardness, etc., cannot be sectioned by other means. For example, thick sections of large embryos are easily made by embedding the embryo in plastic and then making sections at the desired level with a thin hack-saw. The original smoothness of the cut surface is easily replaced by a small amount of grinding and polishing.

In a similar way, sections of bone, teeth and other hard objects can be made. The object is embedded in plastic, thin wafers cut with a fine saw, these wafers affixed to a plastic slide and then ground and polished to the desired thickness.

Two difficulties have been encountered in working with these clear plastics. Each of these will be briefly discussed.

1. It has not been found practical to embed large objects by the technics described in this paper. Two difficulties are encountered in this respect. In the first place, before any wet object can be embedded in these plastics, it must be completely dehydrated. Obviously, dehydration of a very large object presents difficulties. Secondly, it is a difficult task to polymerize large blocks of these plastics without the formation of bubbles in the cast. The polymerization reaction is an exothermic one and if the heat generated in the interior of the hardening mass is not carefully dissipated, this will lead to the formation of bubbles. However, it is possible to polymerize large blocks of the plastic by allowing the reaction to go on very slowly at, or slightly above, room temperature.

2. The hardened plastics are subject to scratching after continued use. This difficulty is easily overcome. Fine scratches are easily removed by rubbing the cast vigorously with silver polish or some other fine abrasive.

LITERATURE CITED

PUCKETT, W. O. 1940 Ethyl methacrylate as a mounting medium for embryological specimens. Science, vol. 91, pp. 625–626.

PLATE 1

EXPLANATION OF FIGURES

1 Seventy-two-hour chick embryo embedded in a thin disk of ethyl methacrylate plastic. × 1.

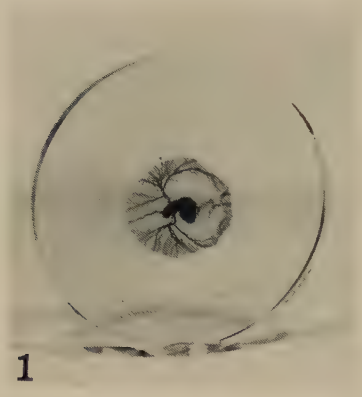
2 Twenty-millimeter sheep embedded in block of ethyl methacrylate plastic. × 1.

3 Twenty-millimeter Amblystoma larvae embedded in disk of ethyl methacrylate plastic. × 1.

4 Dried butterfly embedded in block of ethyl methacrylate plastic. × ½.

5 Frog eggs in various stages of development embedded in a thin disk of ethyl methacrylate plastic. × 1.

6 Seventy-two-hour chick embryo mounted in clear ethyl methacrylate plastic on an unbreakable plastic (Plexiglas) slide. × ¾.



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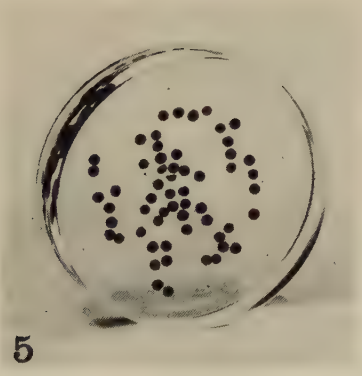

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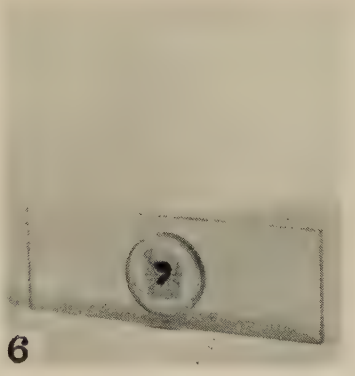

6

EFFECTS OF HYSTERECTOMY AND OF ESTROGEN TREATMENT ON VOLUME CHANGES IN THE CORPORA LUTEA OF PREGNANT RABBITS

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ONE PLATE (FIVE FIGURES)

The corpus luteum is apparently the only part of the reproductive system which is influenced by the uterus. Loeb's ('23) striking demonstration of the effect of removing the uterus on prolonging the survival time of the corpora lutea of guinea pigs remains the outstanding contribution to this problem. In rabbits, likewise, hysterectomy tends to lengthen the luteal survival time by a few days (Asdell and Hammond, '33 and others). The estrous cycles of rats continue unaltered following removal of the uterus (Long and Evans, '22). However, Bradbury ('37) has shown that the pseudopregnant period in operated animals is prolonged about 6 days beyond the average 12 day pseudopregnant period of normal rats; if the uterus is removed during pregnancy (sixth to sixteenth day), "there is a tendency for the corpora to persist for a length of time approaching what would have been term for that pregnancy." Hechter, et al., ('40), recently made the interesting observation that these persisting corpora lutea of hysterectomized pregnant rats undergo rapid involution following implantation of the uterus from a rat in estrus. It is generally agreed that total hysterectomy in women tends to hasten the onset of the menopause whereas this operation does not disturb ovarian function in Macacus rhesus monkeys (Burford and Diddle, '36).

It has often been suggested that the corpora lutea of pregnancy may be sustained at least during some part of their existence by substances arising elsewhere than the pituitary gland and, the placenta has been emphasized as a possible or claimed source of such luteal-stimulating agents. The present work was undertaken to determine what effect the removal of the pregnant uterus (hence all stimuli which may arise from any part of the conceptus or the uterus) would have upon the subsequent length of time that the corpora lutea would survive.

METHOD

The pregnancies were dated from the observed copulation of does on heat. At hysterectomy the uterine tissue was completely removed; the forward resection was made at the uterine end of the fallopian tubes and the other one sufficiently caudad to insure the removal of the entire cervix. The resected end of the vagina was closed with a purse string suture. The fallopian tubes were left intact to avoid producing surgical trauma in close proximity to the ovary. The blood supply to the ovary by way of the ovarian artery was not disturbed and ample evidence was obtained to show that none of the observed ovarian effects could be attributed to diminished circulation.

The uteri were removed from pregnant rabbits, 5, 10, 15, 20 and 25 days after coitus. Usually one ovary was removed at 5 days and the other at 10 days following hysterectomy; thereby, the changes in the size of the corpora lutea during the 10 days subsequent to hysterectomy could be followed. Ovaries obtained from other animals, at various intervals during the gestating period, yielded information concerning the growth of the corpora lutea of pregnancy.

The effect of estrogen (amniotin) on the survival of the corpora lutea following the removal of either the uterus or the hypophysis or both was also determined. These observations were made for the purpose of observing at first hand

the baffling observation that estrogen may prolong the functional existence of the corpora lutea in hypophysectomized rabbits (Westman and Jacobsohn, '37 and Robson, '37), and also to examine the possible relationship of estrogen to the maintenance of pregnancy in rabbits.

The ovaries in most instances were sectioned serially and two maximum diameters (measured approximately at right angles to each other) of each corpus luteum were measured by viewing sections of these bodies against coordinate paper on the stage of a binocular microscope. In some ovaries, especially those in which the corpora lutea were very prominent, free-hand sections were made with a razor and the corpora lutea were measured in a similar manner. From these measurements the volume was determined; the figures thus obtained are subject to an experimental error of approximately $\pm$ 10% as shown by repeated blind determinations.

RESULTS

Following hysterectomy at any time during the first half of pregnancy the growth of the corpora lutea continued up to the fifteenth day after coitus in a manner almost comparable to that seen in unoperated pregnant rabbits during this period (table 1, figs. 1 and 2). The removal of the uterus as early as the fifth day of pregnancy is essentially a conversion of the pregnancy into pseudopregnancy and the subsequent growth of the corpora lutea was similar to that seen in intact pseudopregnant animals. When the uterus was removed on or after midterm, the corpora lutea underwent rapid involution (fig. 3) as shown by the great diminution in volume which occurred within the first 5-day postoperative period (table 1). These degenerative changes were evident upon gross inspection of the ovaries, during laparotomies or at autopsy, by the smallness of the corpora lutea, their whitish appearance (from decreased vascularity), and by their tendency to become submerged into the substance of the ovary as they involuted. Removal of the uterus on the twenty-fifth day of

TABLE 1

Showing the effect of hysterectomy of pregnant rabbits on the subsequent survival of the corpora lutea as determined by measurements of their volume.

RABBIT NO.	HYSTERECTOMY DAYS AFTER MATING	ONE OVARY REMOVED			AUTOPSY		
		Days after mating	Number corpora lutea	Aver. vol. corpora lutea	Days after mating	Number corpora lutea	Aver. vol. corpora lutea
				cu.mm.			*cu.mm.*
68	5	10	4	11.3	15	4	10.5
30	10	15	6	10.7	20	5	4.8
35	10	20	3	5.2			
77		10	4	11.6			
78		10	5	12.3			
46	15	20	9	2.7	25	6	2.1
11	15	20	8	4.3	25	6	2.8
76		15	4	13.0			
45	20	25	8	4.9	30	7	3.0
51	20	25	8	6.6	30	5	4.2
79		20	5	15.3			
86		20	4	14.5			
87		20	7	13.3			
88		20	4	11.6			
65	25	30	9	2.2	35	3	1.2
57	25	30	8	2.3	35	8	2.3
84		23	5	13.8			

pregnancy resulted in the most rapid luteal degeneration that was observed.

EFFECT OF ESTROGEN ON THE CORPORA LUTEA

Hysterectomy during the latter half of gestation apparently deprives the corpora lutea of the source of their stimulus and since estrogen has been shown to be effective in maintaining the corpora lutea of rabbits, it was of interest to investigate this substance as a replacement for the stimulus lost through removal of the pregnant uterus. It was readily demonstrated that the injection of 500 I.U. amniotin daily following hysterectomy on the fifteenth or twentieth day after coitus was completely effective in preventing luteal involution (table 2). After partial involution had occurred (rabbits no. 35 and 60, table 2) estrogen treatment failed to restore the corpora

TABLE 2

Showing the effect of injected estrogen on the survival of the corpora lutea following either hysterectomy or hypophysectomy or both as determined by measurements of their volume.

RABBIT NO.	OPERATION: Organ removed	OPERATION: Days after mating	OPERATION: ESTROGEN DOSAGE DAILY	ONE OVARY REMOVED: Days after mating	ONE OVARY REMOVED: Aver. vol. corpora lutea	ONE OVARY REMOVED: ESTROGEN DOSAGE DAILY	AUTOPSY: Days after mating	AUTOPSY: Aver. vol. corpora lutea
			I.U.		*cu. mm.*	*I.U.*		*cu. mm.*
64	Uterus	15	500	20	11.2	500	25	10.6
27	"	20		20	max. size*	500	30	max. size*
28	"	20		20	" "	500	30	" "
29	"	20		20	" "		30	vestiges*
30	"	20		20	" "		30	"
35	"	10		20	5.2	500	30	4.9
60	"	15		20	7.0	500	25	4.5
71	"	1					4	3.1
72	"	1					4	2.9
80	Pituitary	1		1	0.8	2500	6	5.5
81	"	1		1	1.1	2500	6	5.5
75	Uterus, pituitary	6	500				13	12.6
77	" "	10		10	11.6	2500	20	12.1
78	" "	10		10	12.3	2500	20	12.7
74	" "	23		23	15.6	1000	33	10.0
10	" "	20		20	max. size*	500	29	slightly* smaller

* Ovary not sectioned serially. Estimate of size made upon gross examination.

lutea, although the subsequent involution appeared to progress more slowly than in the absence of estrogen. Since the possibility existed that the estrogen may have been acting upon the corpora lutea indirectly through the pituitary gland, it was necessary to determine its action in animals that were both hysterectomized and hypophysectomized. It will be apparent from an examination of table 2 that the presence of the pituitary is no requisite for this luteal stimulating action of estrogen. In three of five animals the maintenance of the corpora lutea was apparently complete during the 10 day period of estrogen injections and in the other two animals only a slight decrease in luteal volume had occurred. The extensiveness of the surgical preparation involved may have contributed some hazard to complete luteal maintenance.

Having observed the ability of estrogen to sustain the corpora lutea once they were formed, it was of interest to determine the effect of estrogen on the recently ruptured follicles to determine if the corpora lutea would proceed to develop in a normal manner. Accordingly two young adult does (nos. 80 and 81, table 2) were mated and 22–24 hours later hypophysectomized and unilaterally spayed. Daily injections of 2500 I.U. amniotin were started immediately and at autopsy 5 days later, the corpora lutea were prominent, extremely well vascularized and obviously as well developed (figs. 4 and 5) as those of intact does 6 days after coitus. Volume determinations showed an average increase of 550% over the control corpora lutea obtained 5 days earlier (table 2). Smith and White ('31) have shown that luteinization of the walls of the ruptured follicles proceeds in an almost normal manner for 2 days in the absence of the hypophysis but that beyond this limit rapid involution takes place. All subsequent workers are agreed that irrespective of the stage of an established pregnancy or pseudopregnancy a very conspicuous luteal degeneration occurs between the third and fifth day following hypophysectomy.

PLACENTAL EXTRACT

The luteal maintaining influence of the conceptus being thus replacable by estrogen, it appeared possible that in the removal of the pregnant uterus a source of estrogen may have been cut off, and the placenta was suspected as the most likely origin of such estrogen. A batch of 240 gm. of rabbit placentae, taken from does 15–25 days pregnant, was ground and extracted once with acetone and three times with ether. The concentrated lipoidal material was injected into a single spayed adult female rat over a period of 3 days. An estrous vaginal smear was not produced and it may be presumed that placenta does not produce estrogen in sufficient quantities to maintain the corpora lutea of pregnancy.

The placentae of rats yielded a saline extractable substance (cyonin, Astwood and Greep, '39) capable of stimulating

luteal function in rats (Astwood and Greep, '38) but a similar extract of rabbit placenta injected into a rabbit hysterectomized on the fifteenth day of pregnancy failed to maintain the corpora lutea. This animal received 16 gm. equivalent of placenta daily for 5 days and at autopsy on the sixth day the corpora lutea were greatly involuted. The average volume of these luteal bodies was 3.97 cm. (control, 13.02 cm.).

GROWTH OF THE CORPUS LUTEUM

From volume determinations of the corpora lutea obtained at different gestating intervals some information on the normal growth of the corpora lutea of pregnant rabbits was obtained (table 1). These show that the increase in size occurs rather slowly during the first 4 days after coitus as would be expected from the fact that during this time the luteal development is mainly concerned with an encroachment upon the old follicular antrum. From the fourth to tenth day there occurs a very marked increase in volume. The growth curve obtained by Pincus and Berkman ('37) was based on the weights of the corpora lutea of pregnant does and differs from the present data in showing an extremely rapid rate of growth during the first 3 days. It is probable that there is a rapid increase in the actual amount of luteal tissue during this time which would not be apparent from measurements of the ruptured follicles. The source of error in dissecting out and weighing corpora lutea 8 to 62 hours old, however, must be very great. They also found a very slight but gradual increase in the size of the corpora lutea during the last half of pregnancy while the present data indicates that a plateau is reached between the fifteenth and twentieth days.

DISCUSSION

The rapid disappearance of the corpora lutea which follows removal of the rabbit's uterus during the placental phase of pregnancy suggests an extra-hypophyseal source for the luteal stimulus during this time. Robson's ('36 and '37)

observations that the corpora lutea of rabbits do not involute following hypophysectomy in late pregnancy provided the pregnancy is maintained with progestin or progesterone, whereas involution does follow such treatment in early pregnancy, also indicates that the uterine contents (presumably the placenta) aid luteal survival. The follicles, however, underwent involution and soon lost their capacity to respond to an ovulating stimulus (pregnancy urine extract), but this does not prejudice the possibility of an extra-hypophyseal gonadotrophin (stimulating only the corpora lutea) as this author claimed. Additional evidence of the operation of a mechanism for luteal maintenance supplemental to the pituitary during all, or a part, of the placental phase of gestation in other animals, is seen in the work of Pencharz and Long ('33), Selye, Collip and Thomson ('33) (rat); Newton ('35) (mouse); Cole, Howell and Hart ('31) (horse); Browne and Venning ('36) (human); Klein ('38) (hamster) and others. Evidence to the contrary is at hand for the rabbit, (Firor, '33 and others) in that abortion and rapid luteal involution follows the removal of the pituitary from pregnant animals; extracts of the placentae of rabbits likewise did not show the presence of a cyonin factor or of estrogen, either of which might operate to sustain luteal function. However, the very limited amount of placental material available has not afforded conclusive evidence.

The stimulating effect of injected estrogen upon the growth, and survival of the corpora lutea of rabbits is undeniable, yet there is little convincing evidence that naturally secreted estrogen plays an actual role in the maintenance of pregnancy. Estrogen injected into pregnant rabbits is, in fact, a harmful agent and, depending upon the stage of pregnancy, may prevent nidation (Courrier, '30, et al.) or cause the death and expulsion (with a single injection) of the fetuses (Heckel and Allen, '38). The fact that estrus occurs almost immediately after parturition in rabbits is strong evidence that the terminal failure of the corpora lutea occurred in the presence of increasing amounts of estrogen. In the present work, also, estrogen produced by the graafian follicles did

not preserve the corpora lutea, as for instance when the uterus was removed on the fifteenth or twentieth day of gestation; these animals were observed to come on estrus simultaneously with the degeneration of their corpora lutea. The work of Heckel and Allen ('38) on the prolongation of pregnancy with continuous estrogen treatment is of importance, primarily, in showing the effect of estrogen on the corpora lutea of pregnancy, for the conceptus was dislodged, though not expelled, as soon as the injections were started.

Micale ('40) removed the gravid uterus from five groups of rabbits in early pregnancy and, during the next twenty days, four of the groups were injected with folliculin, gonadotrophic hormone, vitamin E and ascorbic acid respectively. The fifth group, killed 20 days after hysterectomy, received no treatment. Involution of the corpora lutea, observed in the control animals, was prevented by vitamin E and by folliculin. The author concluded on the basis of the characteristics of the mammary glands and anterior pituitary, that these artificially maintained corpora lutea were nonfunctional.

We are yet far from a satisfactory understanding of the factors concerned in the production of ovarian function adequate for the maintenance of pregnancy. Robson ('40) has recently expressed a somewhat more hopeful view in that he believes his evidence "suggests that, in the rabbit at least, once ovulation has occurred the development of the corpus luteum and the maintenance of its activity is controlled by an estrogen." There are at least three lines of evidence, however, which dictate caution in assessing the physiological significance of the luteal-stimulating action of estrogen. First among these is the distressing increase in the instances of overlapping function between unrelated hormones. The adaptive capacity of the organism for drawing upon these secondary functions of its hormones is entirely unknown. Second, the luteal secretion of rabbits has a marked capacity for nullifying the activity of estrogen. Courrier ('30) found that it required 500 to 700 R.U. estrogen to prevent the development of normal prenidatory modification of the endo-

metrium. Also Smith and Smith ('31) observed that the luteal hormone, progestin, facilitated the excretion of estrogen in rabbits. Robson's ('39) observation that progesterone failed to inhibit the luteal-stimulating action of estrogen over a short period appears not to be conclusive. Third, the placentae of several rodents play an important role in maintaining luteal activity, and there is no evidence to show that this effect is mediated by estrogen; in fact, the latter substance has not yet been found in extracts of rodent placentae (for review see Corner, '38). Robson ('40) questions the differentiation of rat cyonin from an estrogen factor, but this objection appears not to be well founded since (1) the crude placental extracts do not induce estrus in spayed immature female rats and, (2) injected estrogen negates the decidual reaction upon which the detection of the cyonin activity is based.

There seems to be a distinct difference in the effects of hysterectomy on the corpora lutea of rabbits depending upon whether the uterus is removed after mating, as in the present work and that of Micale, or at a considerable interval of time before mating (Asdell and Hammond, '33). In the former instance, the corpora lutea do not persist beyond the normal pseudopregnant period whereas in the latter, their life span is increased by 6 to 10 days. Bradbury ('37 b) has suggested that an impairment of the metabolism of estrogen occasioned by the removal of the uterus (Pincus, '37) might result in a longer luteal survival through greater (accumulated) estrogen stimulation. The expected results from the action of this mechanism, were not achieved under the conditions of the present experiments.

SUMMARY

Hysterectomy during the first half of pregnancy in rabbits shortened the life span of the corpora lutea of pregnancy to approximately that of pseudopregnancy (15–16 days). Hysterectomy during the second half of pregnancy initiated a precipitous decrease in the size of the corpora lutea. Estrogen injections prevented this luteal involution and similar

experiments in animals that were also hypophysectomized showed that this effect was not mediated through the pituitary gland. Estrogen also stimulated what appeared to be normal corpus luteum development in rabbits hypophysectomized about 12 hours after ovulation.

Extracts of rabbit placentae did not show, in preliminary tests, either estrogenic or cyonin activity.

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PLATE 1

EXPLANATION OF FIGURES

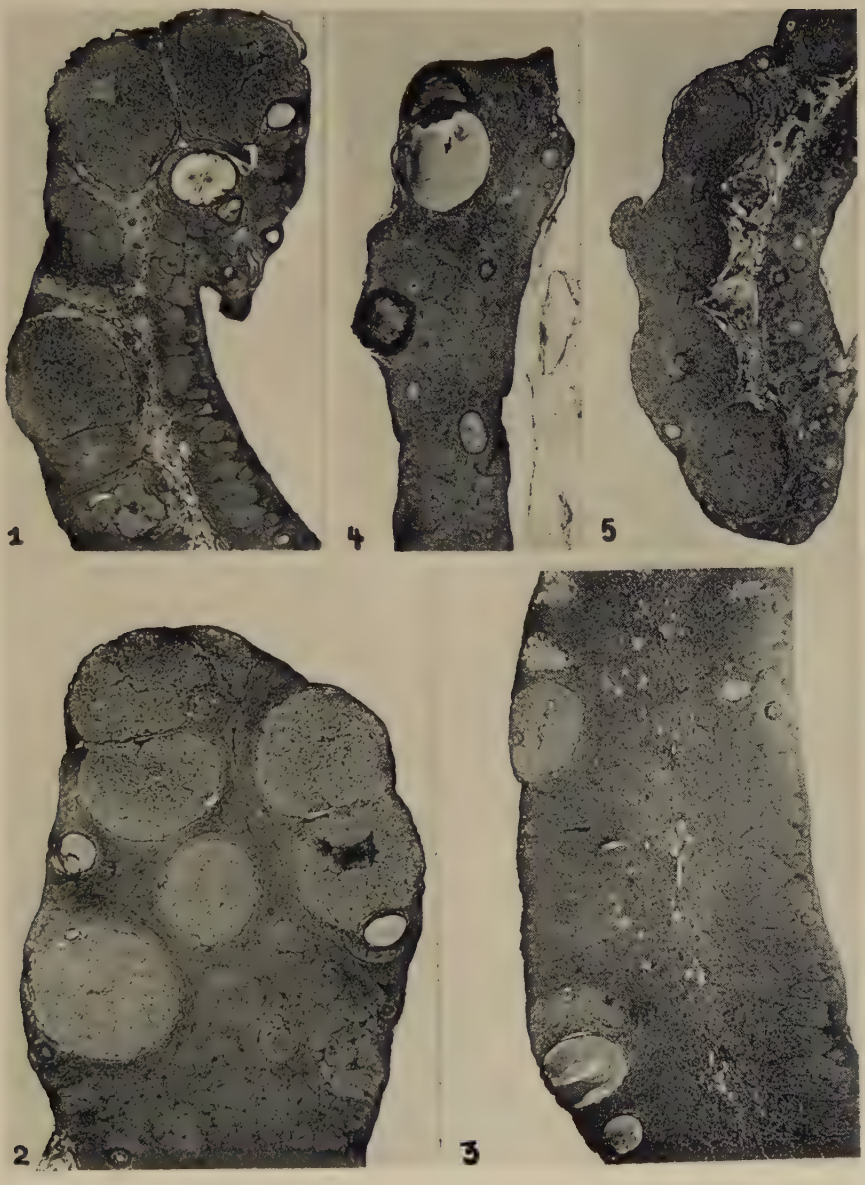
1 Ovary of rabbit no. 68 showing the corpora lutea 10 days after hysterectomy which was performed on the fifth day of gestation. × 7.3.

2 Ovary of rabbit no. 30 showing the corpora lutea 5 days after hysterectomy which was performed on the tenth day of gestation. × 7.3.

3 Ovary of rabbit no. 46 showing the largest corpora lutea remaining 10 days after hysterectomy which was performed on the fifteenth day of gestation. × 7.3.

4 Right ovary of rabbit no. 80 removed at laparotomy 22 hours after mating. × 7.3.

5 Left ovary of rabbit no. 80 showing growth of the corpora lutea induced by estrogen treatment in the 5 days following hypophysectomy which was also performed at the time the left ovary (fig. 4) was removed. × 7.3.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SIZE OF THE PARATHYROID GLANDS OF ALBINO RATS AS AFFECTED BY PREGNANCY AND CONTROLLED DIETS

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ONE FIGURE

The size of the parathyroid is known to vary with diverse experimental factors. The present paper will attempt to show in a semi-quantitative manner the absolute weights and body weight ratios of parathyroids from male and virgin female rats and then compare the effects of pregnancy under diets of controlled calcium and phosphate content. This is the morphological aspect of a series of experiments conducted in collaboration with M. Bodansky and V. Duff.[1] Part of the work has already appeared and the reader is referred to Bodansky and Duff ('39 and '41) for details of feeding and care of the colony and of characteristic reactions to the various synthetic diets.

MATERIALS AND METHOD

Diets. Normal values for weight of parathyroid glands were established on a diet chosen from the list of Cox and Imboden ('36). They found their diet 7 to be optimum for reproductive success in normal animals and Bodansky and Duff confirmed this. We summarize the various diets we employed, designating them by the numbers of Cox and Imboden.

[1] The experimental work is conducted in the John Sealy Memorial Laboratory. Microscopic work was carried out in the laboratory of Histology and Embryology.

Diet 26, with low values for both calcium and phosphate, is so severe that reproduction is difficult even in normal animals and in parathyroidectomized rats it is impossible without some diet modification during early gestation. Most of the work here reported was based on diets 7 and 26 with a few examples from each of the others.

Microscopic technique. In the rat there are but two parathyroid glands and they are quite constant in position. In the living rat the poorly vascular gland contrasts with the dark thyroid. It frequently lies in a fork of the inferior thyroid vein or the vein forms one boundary. Sometimes it

TABLE 1

Synthetic diets uniform except for calcium and phosphate content.

Diet #	%Ca	%P	Ca/P	
7	0.490	0.490	1.0	Adequate for reproduction
8	0.490	0.750	0.66	Phosphate high
10	0.490	2.450	0.2	Phosphate excessive
12	0.735	0.450	1.5	Calcium high
16	1.225	0.245	5.0	Calcium high, phosphate low
19	1.225	1.225	1.0	Balanced at a high level
26	0.017	0.245	0.07	Unbalanced at a low level
27	0.122	0.245	0.5	More balanced than 26

projects from the thyroid surface with the ventral pole embedded but more frequently it lies flush with the surface showing its two greatest diameters. The gland is ellipsoidal to spheroidal. Parathyroidectomy necessitates cutting a large vessel and unless special precautions are taken the gland will be distorted or crushed. It is also necessary to remove some thyroid tissue. The study of the glands, aside from a statistical comparison of groups, was also used to check the completeness of parathyroidectomy, gland normality, and any wide deviations in blood calcium and phosphorus determinations in individual rats used by Bodansky and Duff.

Parathyroid glands of males and virgin females were removed surgically. Those of the pregnant females were fixed in situ on the larynx and were later excised together with

some thyroid tissue. By lighting the tissue from below the parathyroid appears as a more opaque body against a somewhat translucent thyroid. Parathyroid and thyroid block were outlined by means of a camera lucida. The fixing reagent was 10% formalin plus 1% formic acid. Tissue was not left long in fixing fluid though neither maceration, swelling, nor shrinkage occur and the tissues stain well. We used dioxan for dehydration and paraffin for infiltration, cutting serial sections at 15 μ. By orienting the block in sectioning and staining as in previous outlining we drew new outlines over the old and measured shrinkage. Repeated trials of this kind shows our dimensional shrinkage to be about 22%, so that a tissue volume of 1000 has been reduced to 580. This is much less than the 40% dimensional shrinkage reported by Luce ('23). Specific gravity of the blocks as determined by the sinking method in glycerol-water solutions is about 1.04.

Sections are projected and outlined on a bond paper, leaving out large blood vessels and connective tissue masses. Median sections vary from 10 to 100 or more square inches in area and each series includes 40 to 100 sections. An electric perforator is used to cut them out. They are then stacked and weighed. Our formula for reconstruction makes each gram of paper equal to .02 mg. of gland. The paper-weight method has been discussed by Scammon and Scott ('27) and others. Its accuracy, aside from microtechnical difficulties, depends on size of projection, accuracy of focus, closeness of outlining and cutting, and uniformity of paper used. Blumenfeld and Rice ('38) felt that they could use every tenth section. After several trials with irregular or particularly thin or small parathyroids I decided to use every section. Repeating the drawing and cutting produced weight variations up to 4%. Those who have attempted direct weighing of these small bodies always obtain values three to four times those obtained by reconstruction. Swinyard ('39) shows that direct volumetric determinations are inaccurate even if it were possible to make clean dissections. Planimetric

methods also were tried and were found to be more tedious without any evident increase in accuracy.

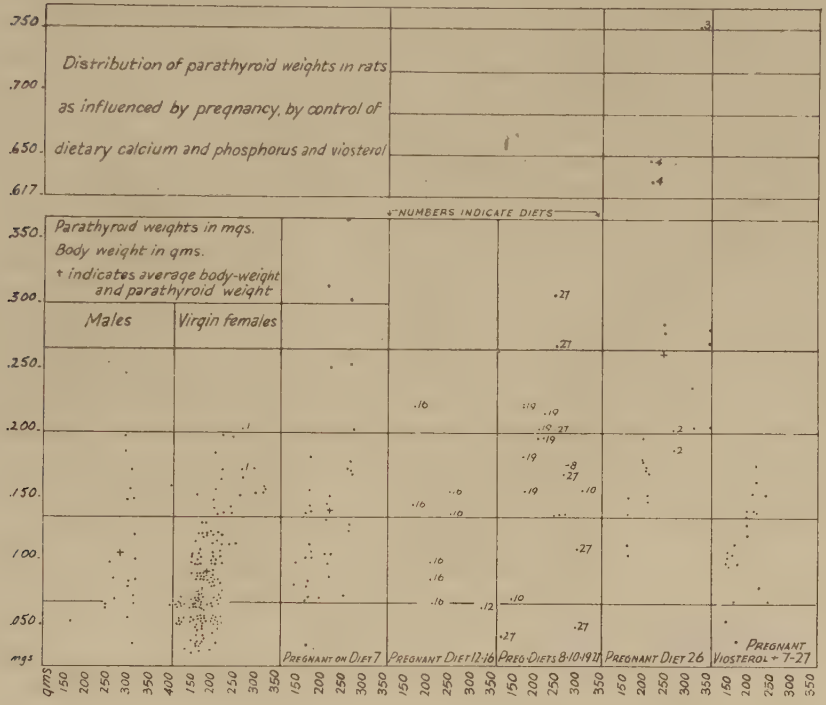
Losses. The surgical removal and technical handling of such minute masses in large numbers is difficult and some losses result. The supposed parathyroid may be a lymph node, or a fat body which disappears in fat solvents or heat. It may be crushed. It may be deeply embedded or it may lie free and be missed. More rarely it may be absent. Sections are sometimes folded or washed from the slides, making accurate reconstruction impossible. A dull knife condenses areas. The number of such faults in our series is not large. We use no parathyroids except in complete series and consequently our pairs are sometimes broken.

WEIGHT OF GLANDS

Males on a good diet. The data have been assembled into a scatter chart for discussion. Single parathyroid gland weights are distributed according to body weight and in some cases they are linked in pairs for special significance. Pairs in general vary together both in normal and in experimental animals though there is almost invariably some difference in members of a pair. Right and left sides show no consistent asymmetry. Out of forty-three pairs the left was larger twenty-two times and the right twenty-one times. The average ratio of small to large was .79 with a standard deviation of .12. We believe that the data are nearly as significant using single glands as in using pairs and it permits including animals from which only one parathyroid could be reconstructed. Even when only one parathyroid could be found it was not large but ranged with the paired glands. This indicates that the other member of the pair was present but was missed.

Fourteen males yielded twenty-eight parathyroids averaging 0.112 mg. each. There is no correlation with body weight, some of the heaviest animals having small parathyroids. The largest pair, occurring on a small animal, was very dense and so unusual, the nuclei being closely packed, that

identification was difficult. They were both alike and there was no adequate reason for discarding them. Average weight curves by both Luce ('23) for the rat and Gilmour and Martin ('37) for man show an adult plateau independent of body weight. Variation at any age makes parathyroid body weight correlation very low. It is higher for the growing



embryo and young animal. The wide variation found needs explanation. It is either functional or genetic in origin. Hammett ('22), working with two strains within the Wistar strain, found great differences in reaction to parathyroidectomy (15% and 79% death in 48 hours respectively), and explained it by genetic differences. While we have had no such death rate, we are trying to reduce or eliminate this factor in future experiments. We believe it plays a part in our present series where it was not suspected. Freuden-

berger and Clausen ('35) found that an inbred strain of Wistar rats isolated from the parent colony for a long time had developed a quite different thyroid structure. Brown, Pearse and Van Allen ('23) remarked that seasonal and functional variation of all the endocrines makes a body weight correlation unlikely. The idea of a seasonal variation in serum calcium in pregnant women has been explored by Mull and Bill ('34) and by Bodansky and Duff ('39) who found a positive but delayed correlation to hours of sunlight, operating possibly through vitamin D production. It is evident that the action of these complex factors make a study of average or standard deviations in parathyroid weights yield little that is not evident in the scatter chart. However, we present a tabulation of all the data.

TABLE 2

Structure of the parathyroid glands of albino rats under the stress of pregnancy and control of dietary calcium and phoshate. Two diets have viosterol added.

Sex	Condition	Diet	Avg. wt. in gms.	Rats	Glands	Gland avg. wt. in mg.	Avg. dev.	Stand dev	mg/Kmg	Sample nuclear count
♂	Mature	Biscuit	278	14	28	.112	.042	.048	0.98	721
♀	Virgin	7	174	100	168	.086	.031	.036	0.99	776
♀	Pregnant	7	218	21	39	.141	.048	.068	1.31	660
♀	"	7v	192	11	15	.117	.029	.034	1.22	
♀	"	26	240	13	18	.265	.141	.200	2.20	468
♀	"	19	193	4	8	.197	.014	.020	2.05	
♀	"	27	257	5	7	.191	.060	.070	1.48	
♀	"	16	199	5	8	.124	.037	.046	1.05	
♀	"	12	327	1	1	.065			043	
♀	"	10	305	1	2	.158			1.04	
♀	"	27v	292	1	2	.122			083	

Virgin females. One hundred virgin female rats gave 168 parathyroids which are more closely grouped than the male glands about the 0.086 average value and show a slight correlation with body weight by sloping to the right in the chart. The virgin females were mostly within the group still growing. The average is a little lower than that of males; and the range, considering the larger number, is certainly less. The large pair, no. 1 in this group, came from an individual that died shortly after operation. It was

probably more dependent on its parathyroids than most of the others. If we relate the average parathyroid weights to average body weights we obtain nearly identical values; 0.97 mg/Kg for males, and 0.99 mg/Kg for females. Younger rats in general have proportionately larger parathyroid and other glands. In absolute values the male is 31% above the virgin female. Since the males were generally heavier than the females the difference may not be significant. Listing parathyroid weights without reference to pregnancy the following workers obtain higher values for females than for males: Morgan ('36), Blumenfeld and Rice ('38), Chang and Chen ('40), and Gilmour and Martin ('37, in man). Pappenheimer and Wilens ('35) state that this difference has no relation to pregnancy. Our absolute values are lower than those of Chang and Chen who give an average of 0.5 mg. for two glands, but we obtain nearly the same weights as Pappenheimer and Johnson ('38) and Bergstrand ('22). Hunter ('37) states that in man the parathyroid body weight ratio is 1.5–6.0 mg/Kg. Gilmour and Martin ('37) give the figure for man as 1–1.5 mg/Kg. Grant and Gates ('24) state that for the rabbit it is 5–5.7 mg/Kg. Eisler ('38) gives about half this value. Using the same methods on human parathyroids that we employed on the rat we obtain absolute values very much like those of Gilmour and Martin. If our results are comparable it appears that the rat lies below the rabbit and man in parathyroid body weight ratio. This correlates with the fact that the rat is not very much affected by parathyroidectomy.

Pregnant rats. Thirty-nine parathyroids from twenty-one pregnant rats on diet 7, chosen to be adequate, show an average weight elevated 65% and the range of variation also increased. If gland enlargement indicates dietary deficiency, diet 7 is deficient. The rats with the largest parathyroids had been through more than one pregnancy and two rats with the largest parathyroids were found sterile when sacrificed after several pregnancies. The data show that pregnancy enlarges the parathyroid and that repeated pregnancies

give a cumulative result. The first pregnancy enlarges the gland from .084 to .114 mg. and the second boosts it again to .208 mg. Bodansky, Campbell and Ball ('39) showed that serum calcium is lowered 4% in women, on the average, during the final months of pregnancy. A similar drop is found in the rat. Carlson ('13) noted the more pronounced reaction to parathyroidectomy in pregnant cats and stated that young animals were more violently affected. This greater dependence of pregnant animals on their parathyroids is evident in rats, particularly near the time for delivery (Bodansky and Duff, '41). Seitz ('33) gives a few data for man, indicating a gland increase in pregnancy. Crotti ('38) (data not given) quotes Churchill as including pregnancy among the functional factors producing an increase. Pappenheimer and Wilens ('35) state that in man pregnancy, single or repeated, produces smaller glands than in nulliparas.

Effects of diet plus pregnancy. The effect of diet variation superimposed on pregnancy shows in the next column. On diet 7 the serum calcium is 9.1 mg. and phosphorus 4.67 mg/100 cc. On diets 12 and 16 six rats have parathyroid sizes below the average for pregnant rats on diet 7. This is in line with Bodansky and Duff ('39) who find that on diet 12 the serum calcium is raised to 9.36 mg/100 cc. and on diet 16 it is raised further to 11.77 mg. Serum phosphate level for diet 16 is very low. Our one case on diet 12 is insignificant. The data on diet 16 are definitely in line with expectation. Diet 16 is rachitogenic to growing rats, probably because of its low phosphorus content because phosphate is as necessary to bone formation as is calcium. Diets 10, 19, and 27 gave successively larger glands. In the group on diet 27 the largest pair of glands is again from a sterile female following her fourth pregnancy. The result cannot be explained on the basis of lowered serum calcium alone because the average serum calcium level for diet 10 is 9.88 mg., for 19 is 8.09 mg., and for 27 is 9.14 mg. Phosphate levels are slightly raised for all of these diets. Our results

for the combined group show better correlation with serum phosphate than with calcium.

When the extremely inadequate diet 26 is superimposed on pregnancy, eighteen parathyroids from thirteen rats average three times the virgin size and nearly twice the size of those from rats pregnant on diet 7. These glands bulge so beyond the thyroid margin that they are stripped off with the crico-hyoid muscle unless special care is taken to conserve them. One large pair, no. 2 on the chart, came from a sterile female after three pregnancies. At autopsy she was found to have a large nephrogenic cyst. The largest single parathyroid, no. 3, came from a rat in its sixth pregnancy and the next largest pair, no. 4, from one in the fifth pregnancy. We are now conducting experiments to dissociate the effects of diet from those of single or multiple pregnancies. An examination of the smallest pair of parathyroids shows atrophy of both glands, a condition very rare in the parathyroid at any age. This pair may have gone through a cycle of enlargement followed by atrophy. Jackson ('16) found this to occur in prolonged inanition. Nonidez and Goodale ('27) found it under severe lack of both vitamin D and sunlight. Luce ('23), feeding a deficient calcium diet shows an enlargement of the same magnitude as ours on diet 26. Her absolute values are higher than ours. Chang and Chen ('40) obtained an enormous peak value of 11 mg. for the rat parathyroids on a deficient vegetarian diet. In both of these cases no account was taken of pregnancy.

Diet 26, very deficient in calcium and low in phosphate, represents the most extreme diet used and the resulting glands were the largest seen. Serum calcium, averaging 8.18 mg., was not below that for diet 19; and phosphorus, while below normal, was not as low as on diet 16. It is evident that no exact correlation can be made with either calcium or phosphorus levels alone.

In certain animals raised on diets 7 and 27 we attempted to inhibit the gland enlargement whether due to pregnancy or diet by feeding viosterol. The dose was from 100 to 400

units daily. Rats on diet 7 received a little more total viosterol than rats on diet 27. The result is seen in the last column of the chart. The parathyroids do not enlarge to the extent they did on diets 7 and 27 plus pregnancy but they are not maintained in the virgin state. The spread of variation is sharply reduced but the difference due to diet is still seen. Shelling ('32) showed that viosterol could produce hypercalcemia even after parathyroidectomy and independent of diet. This makes it somewhat equivalent to diet 16. Albright, Sulkowitch and Bloomberg ('39) clearly differentiate between the action of vitamin D promoting calcium absorption and parathyroid hormone increasing phosphate elimination.

MECHANISM OF GLAND ENLARGEMENT

Gland form and cell size. The normal form of the gland in non-pregnant animals on an adequate diet is ellipsoidal with three axes of different length, the shortest being at right angles to the thyroid surface except when the parathyroid protrudes like a shelf from an embedded base. Every enlargement produced, whether by pregnancy or diet, brings an approximation to spherical form by an increase of the short axis. Once it has attained a spherical form any increase extends all diameters. The first increase affects the cells of the exposed portion. Each cell swells, increasing the proportion of cytoplasm to nucleus. In dehydration, large and small glands shrink proportionately so the swelling is not edema. The lighter color of the projected sections is due to wider nuclear spacing. There is at first a gradation in this spacing from a dense base to a less dense body. The gradation is obliterated as the gland becomes larger. There is in general no marked increase in vascularity nor congestion of existing vessels. I have not seen the varieties of cell type and of cellular pathology described by Kurokawa ('25). There is no marked increase of connective tissue. The cytoplasm remains basophil and homogeneous and all cells appear normal.

Nuclear counts. This mode of enlargement is not accompanied by any flare of mitosis. Jackson ('16) found neither mitosis nor amitosis in glands enlarged by starvation. Rosof ('34) says that there is no cellular increase in the adult parathyroid. If the enlargement is hypertrophy (cell growth), rather than hyperplasia (cell multiplication) in sections of standardized thickness it should be possible to count the nuclei in projected sample areas. As we did not anticipate this problem our 15 μ section proved a little difficult to handle. By using a 3 mm. Leitz objective with a correction collar and a no. 0 ocular, and projecting about 26 inches, the nuclei became separated clearly enough to be marked on white paper with a black lithographic pencil. Nuclei at successive levels appeared by shifting the focal plane and were marked. Every nucleus was recorded in an area 3×5 inches. Then by cross checking with a red pencil the number in this standardized area was counted. We feel that the low counts between 400 and 550 are quite accurate. They become less accurate up to 700 and above that we are certain that many superimposed nuclei are missed. The counts may not therefore be used for strictly quantitative comparison but they are enlightening. The average nuclear count for males was 721 and for virgin females was 776. The male glands are generally more darkly stained and more difficult to count. The normal (diet 7) glands of pregnancy averaged 660 nuclei and those produced on diet 26 averaged 468. The results are certainly in line with the concept of simple hypertrophy for the acute enlargement. Granting that our counts are approximate there must be a nuclear increase in chronically enlarged glands, otherwise the average gland with a count of 660, when doubled in volume, should give a count of 330. The term hyperplasia has been commonly used for parathyroid enlargement without accompanying quantitative data. The published pictures of Grant and Gates ('24), Castlemen and Mallory ('37), and others, give the impression of hypertrophy rather than hyperplasia though the latter term is used in the description. Thompson and Collip ('32), without

quoting a reference, state that recent workers find the gland enlargement to be simple cell hypertrophy.

Factors causing gland enlargement. When we know the mechanism by which the parathyroid gland operates we may determine how it becomes adaptively reduced or enlarged. It is probable that factors having nothing to do with its normal mode of action may be responsible for its size in tumor formation. To all appearances the cells show complete intact cell membranes, yet the potent extract produced by Collip ('25) is a protein colloid which does not pass a collodian membrane. Vassale ('05), Hoskins and Snyder ('27 and '33), and Canterow, Stewart and Housel ('38) say that it does not pass the placenta from fetus to mother but may pass the other way. It is rapidly destroyed by pepsin or trypsin.

The idea that the hormone facilitates protein metabolism, particularly guanadine, advanced by Sharpey-Schafer ('24) was disposed of by Greenwald the same year. Park ('23) showed that both magnesium and phosphorus in serum yielded large non-diffusible fractions and that there were parallel increases during tetany. Huffman, Rebman, Winter and Larson ('30), and Krust, Orent and MacCallum ('33) produced hypercalcemia, irritability and defects of ossification in calves by magnesium poor diets and Shelling ('35) showed that injection of magnesium will elevate serum phosphate. However, I find no statement that parathyroid hormone is related to magnesium levels.

Calcium and phosphate maintain a reciprocal relation in serum and the ion product constant must reach a certain minimum value to permit ossification. In renal insufficiency, experimental or otherwise, Pappenheimer ('36) and Pappenheimer and Johnson ('38) found that the gland is enlarged. In this case phosphate is retained and calcium is reciprocally reduced. A similar result was obtained by Drake, Albright and Castlemen ('36) by parenteral phosphate injections, and by Hertz and Kranes ('34) in the rabbit from pituitary injections. Jackson ('16) found enlarged glands as a result

of inanition. Gamble, Ross and Tisdale ('23) showed that in fasting ketosis was accompanied by predictable losses of sodium, potassium, and magnesium but that the loss of calcium was ten times expectation and the phosphorus loss was also high. Eisler ('38) found that hydroxylamine injections in rabbits caused parathyroid hyperplasia without apparently altering either bone structure or kidneys but resulted in marrow atrophy. Shelling, Asher and Jackson ('33) note that injection of parathyroid extract also reduces bone marrow. Considerable calcium shift can occur within bones or between bones and soft tissues without being easily visible. Nonidez and Goodale ('27) enlarged the glands of chickens by a deficiency of both vitamin D and sunlight. Grant and Gates ('24) by ultraviolet radiation in rabbits obtained an increase of 180% in gland size.

Calcium levels in serum may be kept above normal and the parathyroid gland will be reduced below normal. We accomplished this by diet 16 in which serum calcium is kept high and phosphate so low that the diet is rachitogenic for growing animals. Jaffe and Bodansky ('30) produced a similar condition in dogs with massive injections of parathyroid extract. They reduced the glands one-half to one-third below normal and in prolonged treatment withdrew much calcium from bones, producing osteitis fibrosa. Burrows ('38) had similar experience with rats, finding that the gland cells failed to multiply. DeRobertis ('40) found cytological effects in 6 hours. Canterow, Stewart and Housel ('38) found no effect after 72 hours even though the serum calcium rose to 22 mg/100 cc. MacJunkin, Tweedy and McNamara ('37) show that subcutaneous injections of calciferol, while elevating the blood calcium, cause bone resorption in rats within 48 hours and that parathyroid extract injections will do the same without disturbing the serum calcium level. The mere statement of serum calcium level does not indicate the rate at which it is being eliminated by either kidney or intestine. The size of the parathyroid gland indicates a compensatory adaptation to withdrawal of calcium from reserves and seems

to be unaffected by intake or elimination. Since low calcium always results in loss of reserves the glands enlarge under these conditions. An average lowering of serum calcium by 4% during the last half of pregnancy may not indicate the strain of the homeostatic system for calcium. The parathyroid gland attempts to compensate by enlarging and producing more hormone but at least in the face of a high phosphate level it fails. Hoffman ('33) said that there is no free parathyroid hormone in the blood of non-pregnant animals, that there is free hormone during pregnancy and a decline before the end of the cycle. Gland activity, therefore, increases before the serum calcium drop which indicates its failure. The cumulative effects of pregnancy in rats is probably based on the close succession of pregnancies without recovery. Calcium loss from reserves attacks the last deposited calcium which is most easily soluble. This preosseous border is readily made visible, in sections of teeth and bone fixed in potassium bichromate, even after decalcification. After this soluble deposit is gone the gland may continue to enlarge without causing a comparable increase in the calcium loss.

The close relation of gland size to calcium metabolism is shown by Howard ('40) who removed a parathyroid tumor and lowered serum calcium from 19 to 10.2 mg/100 cc. in 9 hours. Borchers ('19) found that grafting one gland in man was adequate to obviate symptoms of tetany. Rössle ('38) found that a newborn infant, dying in tetany, was lacking parathyroids, which might mean that the mother's parathyroid's had supported it until birth. We do not know the meaning of strains of animals with large or small parathyroids except that in the rabbit the glands are reported to be relatively large and the serum calcium level is also normally high. I find rabbit parathyroids very difficult to locate for measurement.

Compensatory hypertrophy after partial parathyroidectomy does not occur according to Rosof ('34). Failure in our own series of rats to alter the blood calcium levels by removal of one parathyroid supports the idea and yet it is

difficult to correlate these findings with the evident sensitivity of the glands in responding to pregnancy. Rosof limits active cells to those reducing osmic acid, or to about 25%. This may mean, not that three-fourths are inactive, but rather that the synthetic phase of endocrine secretory activity takes about three-fourths of the time so that one-fourth are in the excretory phase. The cells in hypertrophy react uniformly throughout the gland. One must always keep in reserve the possible suggestion that lack of calcium may act on the parathyroid like a lack of iodine on the thyroid, producing useless enlargement. The parathyroid is not a storage organ for calcium but taken in combination with bones it may have a similar pattern. I recently found a human parathyroid of 620 mg. and a second of 80 mg., both hyperplastic and associated with a medium-sized colloid goiter and with skeletal losses. I do not believe the parathyroid problem can be completely understood without a simultaneous study of the thyroid because the thyroid hormone affects calcium metabolism and parathyroidectomy alters basal metabolism.

As far as we have observed, the glands of both sexes vary together under similar diets, though Chang and Chen ('40) state that on identical very poor vegetarian diets the female rat has a higher blood calcium than the male, and the male parathyroid enlarges to twice the size of the female gland. This is not what would be expected.

It appears that the parathyroid gland grows in response to a deficiency of blood calcium, however produced, or to the rapid utilization of parathyroid hormone in accelerated calcium metabolism whether it draws upon fresh supplies or skeletal reserves.

CONCLUSIONS

1. Functional variations of the parathyroid gland outweigh variations due to sex, weight, or right and left asymmetry.

2. The parathyroid glands of male and virgin female albino rats have approximately the same adult weight and body

weight ratios. The average is about 0.99 mg/Kg and is a little lower than that for man.[2]

3. Pregnancy in the rat on a diet adequate for reproduction produces a simple hypertrophy of about 65% involving both glands uniformly. In repeated pregnancy the hypertrophy is cumulative.

4. A diet high in calcium and low in phosphate, while not permitting optimum reproductive success, keeps the serum calcium level high, phosphorus low, and leaves the parathyroid gland at or below normal size.

5. Parathyroid hypertrophy of pregnancy may be aggravated to about twice the normal pregnant size by means of a diet extremely inadequate in calcium and low in phosphate. Diets with lesser degrees of inadequacy or containing much phosphorus give intermediate enlargements.

6. In long continued stimulation of the gland there is additional hyperplasia which may double the cell number.

7. Viosterol in sufficient doses, whether on adequate or inadequate diets, protects the gland to some extent against the stimulus to hypertrophy though it does not entirely prevent its action.

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THE PATTERN OF APPOSITIONAL GROWTH IN THE INCISOR OF THE RAT [1]

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FIVE FIGURES

INTRODUCTION

In 1935 Steadman and Schour studied the pattern of appositional growth of the incisor of the rat and assigned to that tooth the form of an Archimedian spiral. The form is that of a cylinder coiled upon itself, thus having a spiral angle of 90° (fig. 1). The rate of growth has been variously estimated. Marshall ('20), on the basis of vital staining, stated that the dentin of the rat incisor grew at the rate of 10 μ per day. Schour and Steadman ('35) recorded an average daily apposition of 16 μ in both the enamel and dentin, but found no gradients of growth. In 1939 Schour and Hoffman gave injections of sodium fluoride and alizarine Red S to a large number of rats and again found an average rate of growth of 16 μ per day.

However, in view of the general laws of growth elucidated by D'Arcy Thompson ('17) and Huxley ('32) it would appear that the rat incisor should be characterized by a gradient of growth similar to that seen in other organs and should have a form resembling the logarithmic spiral (a cone coiled upon itself—fig. 1). Accordingly, the purpose of this study was to determine whether such growth gradients do exist in the incisor of the rat; to study its geometric form; to trace its incremental pattern at various ages; and to measure the appositional life-span of the formative cells of the dentin.

[1] This work was aided in part by a grant from the Carnegie Corporation of New York.

The methods used were those employed by Massler and Schour in their study of the life-span of the formative cells in the human teeth.

METHOD AND MATERIALS

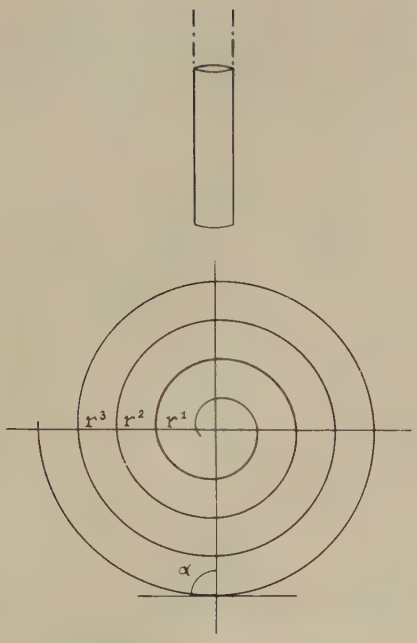
Growth gradient of dentin apposition. Sixty rats were injected with alizarine Red S at different ages and at varying intervals of time. This dye stains the incremental layer of dentin forming and calcifying at the time of the injection. The rate of apposition was obtained by measuring the distance between two lines and dividing it by the number of intervening days.

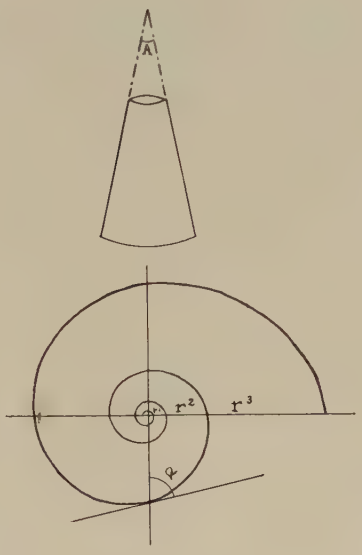
Spiral and apical angles. The spiral angle is the angle (α) between the radius vector (r) and the tangent to the curve (fig. 1). The spiral angle of a tooth which represents the degree of its curvature is indicated by the angle between the dentinal tubules and the dentino-enamel junction (figs. 1 and 2). It was obtained by projecting ground and decalcified sections by means of a camera lucida and using a protractor.

The apical angle of a tooth is the original angle of the incisal edge before abrasion has occurred. It was determined by constructing from measurements an isosceles triangle representing the length-to-width ratio of the incisor (figs. 1 and 2). The angle opposite the base of the triangle represented the original apical angle. This angle was also calculated by measuring the base angles with a protractor and subtracting their combined angulation from 180°.

Incremental growth pattern. This pattern was reconstructed by projecting by means of a lantern-slide projector ground sections of the incisors of the experimental rats and tracing the normal and experimental (alizarine) lines into a tracing of the incisor at a given age (fig. 3).

Life-span of the formative cells of dentin and enamel. The life-span of these formative cells may be calculated by measuring their total extracellular product (enamel rod or dentin width) and dividing this amount by the daily average rate of apposition (Massler and Schour). Micrometer measure-





ARCHIMEDIAN SPIRAL	LOGARITHMIC SPIRAL
$(r = a^{\theta})$	$(r = a^{\theta})$
Form: Cylinder coiled upon itself	Cone coiled upon itself
Apical angle (A): 0	1° – 180°
Spiral angle (α): 90°	16° – 89° 8′
Increments (r): Equal (Arithmetic progression)	Increase in geometric progression

Fig. 1 Diagram showing differences between the Archimedian and the logarithmic spirals.

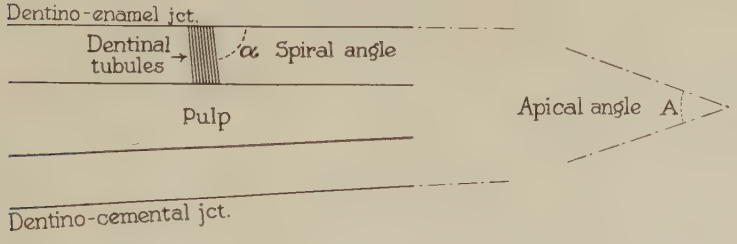


Fig. 2 Diagram showing method employed in order to obtain the spiral and apical angles.

ments were taken of the full width of the primary dentin in the incisors at different ages. Care was taken to select the level of the alveolar crest where the full width of the primary dentin had already been deposited and the odontoblasts (dentin-forming cells) were in an inactive state.

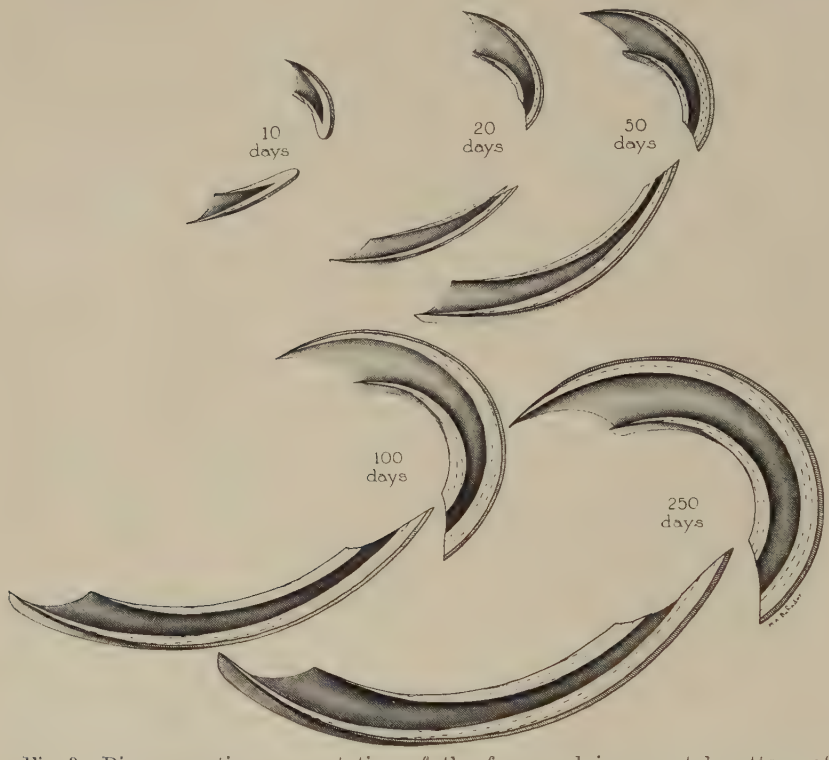


Fig. 3 Diagrammatic representation of the form and incremental pattern of the rat incisor at successive ages, as seen in longitudinal hemi-sections. Note the conical form and increased size of the teeth as the animal becomes older. The incremental pattern of the dentin is indicated by dotted lines.

Measurements were made along the direction of the dentinal tubules (the path of cellular activity). The full width of any particular level of dentin is the product of the daily rate at which its matrix is deposited and the number of days in its physiologically active life-span. After measuring the width of the primary dentin (in μ) and the daily rate, it

was possible to calculate the number of days in the physiologically active life-span of the dentin-forming cells.

OBSERVATIONS

Rates and gradients of appositional growth. The rates of dentin apposition range from 24 μ per day at 10 days of age to 16 μ per day at 40 days of age (table 1). After this time the 16-μ rate of growth per day remains characteristic and relatively constant, indicating the approach of the conical to the cylindrical form (which is a special case of the cone). At this time the dentino-enamel junction and the dentino-cemental junction tend toward parallelism.

Alizarine was not effective in demarcating the enamel, but previous work (Schour and Hoffman, '39) using sodium fluoride as a vital stain showed that the rate of apposition in the enamel and dentin was the same.

Apical angle. Measurements of the apical angle were made from birth to 150 days of age. The angle was approximately 16° with variations from 14° to 20°. These are due to the crudeness of the method which employed pencilled lines and protractors. After 150 days the dentino-enamel and dentino-cemental junctions approach parallelism much more closely than at an earlier age, giving the effect of an apical angle of 4°.

Spiral angle. From birth to 150 days of age the spiral angle was 86° with only minor variations. By means of the spiral constructed in figure 4 this angle was tested and found to be correct. Attempted projections on spirals of 84° and 88° failed.

After 150 days of age the angle varied more readily. Animals from several stocks were used with consistent results.

The curvature of the incisor toward the lingual side is readily visible in labio-lingual sections and is related to the greater growth rate on the labial than on the lingual surface. This conforms with the law of developmental direction (Scammon, '33).

TABLE 1

Total dentin width, daily rate of apposition and estimated appositional life-span of odontoblasts in the incisor of the rat at varying ages

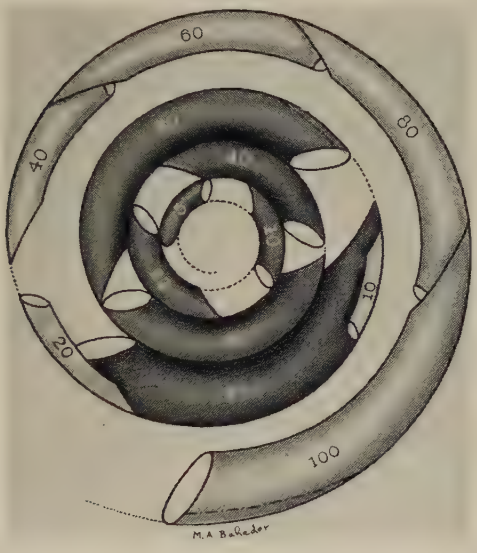
AGE OF RAT IN DAYS	UPPER INCISOR						LOWER INCISOR					
	Labial dentin			Lingual dentin			Labial dentin			Lingual dentin		
	Width [1]	Rate per 24 hours	Estimated functional life-span [2]	Width	Rate per 24 hours	Estimated functional life-span	Width	Rate per 24 hours	Estimated functional life-span	Width	Rate per 24 hours	Estimated functional life-span
10	150 μ	24 μ	6 days	110 μ	24 μ	4–5 days	180 μ	20 μ	8 days	160 μ	18 μ	9 days
17	250 μ	22 μ	11 days	220 μ	22 μ	10 days	255 μ	18 μ	14 days	215 μ	18 μ	12 days
25	300 μ	19 μ	16 days	275 μ	20 μ	13–14 days	335 μ	17 μ	19 days	250 μ	17.5 μ	14 days
30	315 μ	18 μ	17 days	280 μ	18 μ	15 days	340 μ	17 μ	20 days	280 μ	16.5 μ	17 days
40	430 μ	16 μ	27 days	390 μ	16 μ	24 days	420 μ	16 μ	24 days	330 μ	16 μ	20 days
45	460 μ	16 μ	29 days	425 μ	16 μ	27 days	425 μ	16 μ	26 days	340 μ	16 μ	21 days
55–60	540 μ	16 μ	34 days	470 μ	16 μ	29 days	435 μ	16 μ	27 days	420 μ	16 μ	26 days
63	575 μ	16 μ	36 days	530 μ	16 μ	33 days	315 μ	16 μ	20 days	300 μ	16 μ	19 days
75–80	670 μ	16 μ	42 days	635 μ	16 μ	39 days	560 μ	16 μ	35 days	530 μ	16 μ	33 days
90	700 μ	16 μ	44 days	635 μ	16 μ	39 days	370 μ	16 μ	23 days	360 μ	16 μ	22 days
100	775 μ	16 μ	48 days	715 μ	16 μ	44 days	475 μ	16 μ	28 days	405 μ	16 μ	25 days
118	930 μ	16 μ	58 days	745 μ	16 μ	47 days	595 μ	16 μ	37 days	550 μ	16 μ	34 days

[1] Averages obtained by micrometer measurements at the level of the alveolar crest.

[2] Life Span $= \frac{\text{Length of Dentinal Tubule (or total dentin width)}}{\text{Rate of Apposition}}$

Incremental growth pattern. Figure 3 shows the results of tracing the normal and experimental incremental lines upon the outlines of incisors at particular ages and gives the relative size of these teeth.

It is apparent that the dentin is apposed in the form of cones one within the other, and that the cones are curved lingually. The tracings of other rodent incisors (rabbit, guinea pig) show a similar pattern. These stages are selected segments of the complete logarithmic spiral reconstructed in figure 4. This figure represents the total amount of tooth substance that is formed throughout the life of the animal and that would be obtained in the absence of attrition (as in fig. 5).



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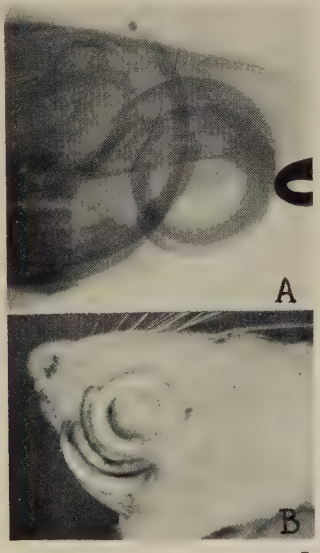

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Fig. 4 Successive segments of upper (dark) and lower (light) incisors of rats of different ages, arranged in a continuous spiral form. Figures indicate days of age. This form would be obtained in the absence of attrition (elongation). Note that the upper and lower incisors are different segments of the same logarithmic spiral.

Fig. 5A Radiograph of a rat in which the spiral elongation of the upper incisors resulted from an experimental fracture of the lower. 5B Photograph of a rat with unworn upper incisors following an accidental fracture of the lower incisors at an early age.

Appositional life-span of odontoblasts. The total width of dentin was obtained by micrometer measurements taken at the level of the alveolar crest. The dentinal tubules range from 150 μ at 10 days of age to 930 μ at 118 days of age, after which they remain relatively constant in length (table 1). The functional life-span of the odontoblasts was found to be greater in the labial (6 to 58 days) than in the lingual (4 to 47 days), and also greater in the upper than in the lower incisors.

Life-span of ameloblasts. The appositional life-span of the ameloblasts showed a much narrower range than did that of the odontoblasts, and increased with the age of the animal (table 2).

TABLE 2

Total width of enamel and estimatel appositional life-span of ameloblasts in the incisor of the rat at varying ages

AGE OF RAT IN DAYS	UPPER INCISOR		LOWER INCISOR	
	Enamel thickness	Estimated active life-span in enamel formation	Enamel thickness	Estimated active life-span in enamel formation
20	60 μ	4 days	90 μ	6 days
30	74 μ	5 days	105 μ	7 days
50	80 μ	5 days	125 μ	8 days
60	80 μ	5 days	120 μ	8 days
75	80 μ	5 days	120 μ	8 days
100	90 μ	6 days	130 μ	8 days
110	95 μ	6 days	140 μ	9 days
120	98 μ	6 days	152 μ	10 days
150	98 μ	6 days	156 μ	10 days
200	98 μ	6 days	155 μ	10 days

The length of the enamel rod of the rat incisor ranges from 60 μ at 20 days of age to 155 μ at 200 days of age, after which it tends to become relatively constant. On the basis of an average daily rate of 16 μ of enamel apposition it was estimated that the life-span of the ameloblasts in the upper incisors ranged from about 4 days in a rat 20 days of age to about 6 days in a rat 200 days of age. In the lower incisor the life-span was greater—from 6 to 10 days. No significant sex or other differences were found.

DISCUSSION

Huxley shows that the logarithmic spiral form must result when a method of appositional growth is combined with any gradient in the growth rate. Since one of the fundamental laws of growth is the fact that it never has a constant, unvarying rate, it follows that all structures formed by accretional growth must be of a logarithmic spiral form. Of course the gradient of growth may be so small as to be almost negligible and the resultant structure will approach the form of a curved cylinder with a curvature that is circular (90°) (fig. 1).

In a previous study of the laws of growth as they relate to the early conical forms of teeth (Herzberg and Massler, '40) it was found that throughout the phylogenetic scale the same laws were applicable. While most conical teeth in even the early forms show the beginnings of the logarithmic spiral form, the rat incisor shows this feature to a marked degree. At different ages the entire tooth is merely a different segment of the same logarithmic spiral (curved conical form). By the same token the lower teeth are found to be just a later segment of the same spiral (fig. 4). The rat incisor is now added to the list of structures growing in this manner.

SUMMARY AND CONCLUSIONS

The rat incisor has the form of a recurved cone whose apical angle is 4° and spiral angle is 86°. Its form is therefore that of a typical logarithmic spiral.

Gradients in the rate of dentin apposition were demonstrated by injections of alizarine Red S and the life-span of the formative cells was estimated.

The rat incisor conforms in all respects to the laws of growth applicable to similar organs.

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THE ARRANGEMENT OF THE CAPILLARY TUFT OF THE HUMAN GLOMERULUS[1]

AN INJECTION STUDY

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FIVE FIGURES

The great importance of the glomerulus as a functioning unit of the nephron is well appreciated but the arrangement of its vascular tuft and the distribution of the glomerular capillaries is not well understood. Marcello Malpighi first described the glomeruli in 1669 in his "De Viscerum Structura" in which he demonstrated by the perfusion of colored fluid through the renal artery that they were attached directly to the vessels. It was about 180 years before the next step in the understanding of the glomerulus was made by the surgeon-anatomist William Bowman of Kings College Hospital in 1842. He described the afferent arteriole as breaking up into two to eight branches which "diverge like the petals of a flower". Bowman was the first to notice that the parenchyma of the kidney is supplied entirely by the efferent glomerular arterioles. In describing the glomerular tuft he noted that the lobes do not communicate except at the root and that the afferent vessels subdivide once or twice as they advance over the surface of the ball. Ludwig in 1872 was probably the first to describe accurately the arrangement of the capillary tuft. He observed that immediately after the entrance of the afferent arteriole into the spherical tuft it divides into four to eight widely diverging

[1]Awarded the annual Phi Delta Epsilon prize in medicine for 1940.

branches. Each branch ramifies and the secondary branches coalesce towards the center of the capsule to form the vas "afferens" (efferens). The capillaries proceed from the primary branches of the vas afferens and reunite into a common veinlet and the vas "afferens" (efferens) is recomposed in the same manner that the afferent trunk divides. When this occurs, Ludwig noted, the glomerulus represents several vascular lobules which are continuous at either end with an artery and a vein. Except for the improper use of the word "vein", this description is essentially correct as it stands. The next important contribution was made by Johnston who in 1899 made a wax reconstruction of a human glomerulus. For the first time the glomerulus was graphically represented in an accurate manner. Johnston described the afferent arteriole as branching into five smaller vessels and he noted that where lobulation appeared it was all superficial. But most important, he found anastomoses between the capillary loops of one lobule as well as between the loops of different lobules. "Thus", he wrote, "the blood in passing from the afferent to efferent vessels has the choice of numerous paths of varying length."

Since Johnston's article numerous investigators have worked on the finer renal circulation and upon the glomerulus. Book in 1936 made a wax reconstruction of the glomerulus to determine the secreting area but did not completely assemble his wax plates. So far as I have been able to determine, this is the only other wax reconstruction of glomerular vessels. R. B. Allen in 1926 reconstructed the lobules (not the vessels) in wax from cases of chronic glomerulonephritis. Injections with gelatin and with celloidin have been carried out by Lee-Brown in 1924, Morison in 1926, Gänssler in 1934 and others. The photomicrographs employed by various authors are good, but obviously cannot display the three dimensional relations of the glomerular tuft; likewise, the descriptions of the glomerulus have not been carried

out in any detail. Apparently there is no detailed accurate picture of the glomular tuft in the literature showing the capillaries as illustrated in figure 1, B.

Although Johnston's reconstruction showed anastomoses, many investigators have failed to find them. Among those who believe anastomoses to be present are Prenant and Bouin, Jordan, Szymonowitcz, Stöhr, Cushny, Renaut, Johnston, Disse, Gosset and Borst. On the other hand, Ludwig, Sappey, Von Ebner, Vimtrup, von Möllendorff, Miller and others believe that no anastomoses exist. Aside from Johnston's work there is no proof of anastomoses in the human glomerulus and Vimtrup has criticised Johnston's reconstruction on this point.

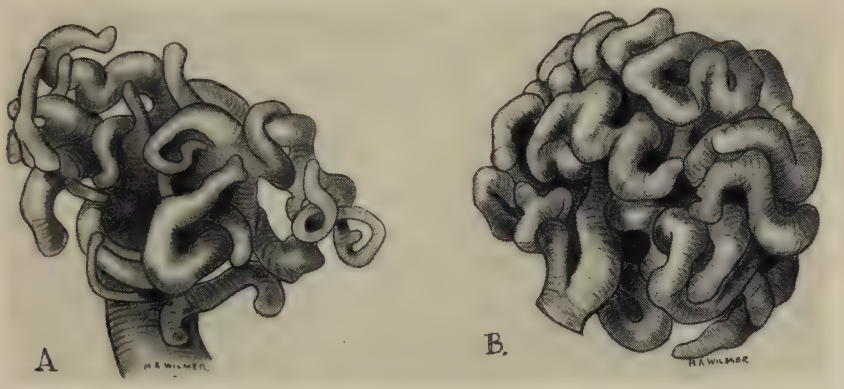

Fig. 1 (A) Partially injected glomerulus viewed from the superior-lateral aspect, and showing the course of the primary branches at the vascular pole.

(B) Completely injected glomerulus showing the afferent arteriole at the lower left, while the efferent arteriole is not seen from this view. The serpiginous non-anastamosing course of the peripheral capillaries is seen. There is a suggestion of the lobular arrangement, though less marked in this specimen than in some others.

Thus, there is a great deal of literature on the arrangement of the capillary loops of the glomerulus but there is only one wax reconstruction. Since variations are almost the rule in vascular anatomy, it was thought desirable to examine as many as possible by a quantitative technique.

METHOD OF STUDY

The celloidin corrosion injection technic was chosen as a method of study, since by injections varying in their penetration, the glomerulus could be obtained and thus show the manner of branching. The injection method has been amply described in the literature by many men, among them Hinman, Morison and Lee-Brown ('23, '24), Barker ('28) and Pettigrew ('34).

Twenty adult and six newborn human kidneys were taken for this study. These were obtained from the postmortem room within 12 hours after death. Half of the specimens were injected for the general circulatory pattern and the remainder for the circulation within the glomerulus.

A cannula was tied in the renal artery and water was perfused through it until the flow from the vein was no longer blood-tinged. After the vascular system had been washed out a celloidin-acetone-camphor solution was injected under pressure. In such a mixture, the acetone combines with the water and the celloidin precipitates out to make a cast of the vessel. The camphor is added to make the final preparation less brittle and alkanin is used as a red dye. After the specimen had been injected and had set for 24 hours under a positive pressure while submerged in cold running water it was placed in pure commercial hydrochloric acid for 24 to 48 hours and the parenchyma was macerated leaving just the celloidin cast. Although thin sections of the injected kidney cleared by the Spalteholz method are valuable, corrosion in hydrochloric acid was found to be most satisfactory.

After maceration, the cast was carefully washed with a thin stream of water under a dissection microscope. The small interlobular arteries were dissected out and cut off with a fine scissors and placed on a glass slide to be observed. First a satisfactory specimen was picked out with the aid of a binocular directing microscope; then drawings were made under low power by the author with a binocular single objective microscope. High power was resorted to when the relation of the finer vessels was in doubt. By using trans-

lumination and carefully rotating a strong light around the stage of the microscope, occasionally using the mirror for through and through illumination, it was possible to determine the relationship of the smaller vessels.

Variations in the viscosity of the solution and the injection pressure used were made in accordance with the extent of penetration desired. A thin (3%) solution was first forced into the renal artery under a sudden maximum pressure, thus reaching the smallest capillaries and making a cast of them. This maximum pressure was maintained for 10 minutes at which time the solutions were changed; then a thick (10%) solution was injected under 350 mm Hg pressure and maintained for 24 hours to make a cast of the larger vessels, the so-called double injection technic. When the 3% solution was injected at 350 to 400 Hg, only the initial primary branches arising at the vascular pole of the glomerulus were penetrated, the injection ending blindly where the fluid stopped. With pressures of 400 to 500 mm Hg a cast of both the primary and secondary branches was obtained and, finally, at 600 to 650 mm Hg the glomerulus could be completely injected as well as the efferent arterioles. Specimens of each type were obtained and studied.

OBSERVATIONS AND DISCUSSION

There are approximately 1,000,000 glomeruli in a single human kidney or a total volume of about 4.2 cc, with the diameter of the loops averaging about 10 micra, as determined by Vimtrup ('28). Book ('36) ascertained that the filtration area of a single typical human glomerulus was 0.3813 sq. mm. Book also estimated that the total length of the capillary loops in each glomerulus was about 25 mm.

The renal circulation has been described in many reports in the literature including those by Broedel ('01), Morison ('26) and Gänssler ('34). The afferent arterioles usually terminate in one glomerulus but frequently in two or occasionally more. This "twining of the glomeruli" was first described by Beer, who noted that the afferent arteriole

divided to bear two glomeruli sometimes within a single capsule; sometimes there were two capsules. Morison and also Lee-Brown noted certain rudimentary glomeruli which arise directly from the arcuate arteries which are peculiar in size and in appearance as well as in the length of their afferent arterioles. These are relatively frequent in the human kidney but rare in other mammals except the cat.

Vimtrup reviewed the literature pertaining to the glomerulus in 1928 and presented his prussian blue experiments. His findings were not at variance with those of Ludwig in 1872. Employing not only serial sections but methods whereby the loops could be separated with a fine glass needle he was able to conclude that there were no anastomoses. He stated, however, that the possibility of anastomoses in the deepest portion might still remain, although he was never able to observe them. In view of the fact that all the parenchyma is supplied by the efferent arterioles, this point seems to be one of the most important ones to settle; for, if anastomoses exist at the vascular pole, especially between the afferent and efferent arterioles, as has been pointed out by some observers, it would mean that obliteration of the most distal capillary loops, as occurs in proliferative glomerulonephritis, would not close off the vascular supply to the parenchyma through the efferent arterioles and would not result in renal ischemia. The question of anastomosis is also important in evaluating the necrosis of the lobules as occurs in embolic obstruction.

Turning now to the description of the injected specimens, it was found that the afferent arteriole branches into from two to ten primary branches at the vascular pole and that these arise in no definite manner or number and show no constant pattern. In general, after a somewhat peripheral arching course they bend back upon themselves in an irregular manner to approach the vascular pole. Occasionally the glomeruli with only two primary branches at the vascular pole appear as two independent vascular tufts, probably a stage just short of the so-called twin glomeruli. Injections

of just the vascular pole and the smaller vessels arising from it (fig. 2) show the variation in pattern. Twin glomeruli are shown in the middle drawing, on the extreme left and an afferent arteriole bearing three glomeruli in the upper right hand corner. Both afferent and efferent arterioles in a completely injected glomerulus are shown at the second from the left in the top row.

Fig. 2 Injections of glomeruli showing variation in pattern at the vascular pole.

The secondary branches vary considerably in number and position in their origin from the primary vessels. In some glomeruli numerous small secondary vessels are given off at almost right angles all along the course of the primary vessels (fig. 3, D; fig. 4) while in others the primary branches are slightly tortuous and give off only an occasional secondary

twig before turning back at the periphery, though both extremes exist. A partially injected glomerulus viewed from the superior aspect (fig. 3, C) shows the arrangement of the primary branches to be essentially peripheral and possessing graceful arches. In the incompletely injected vessels

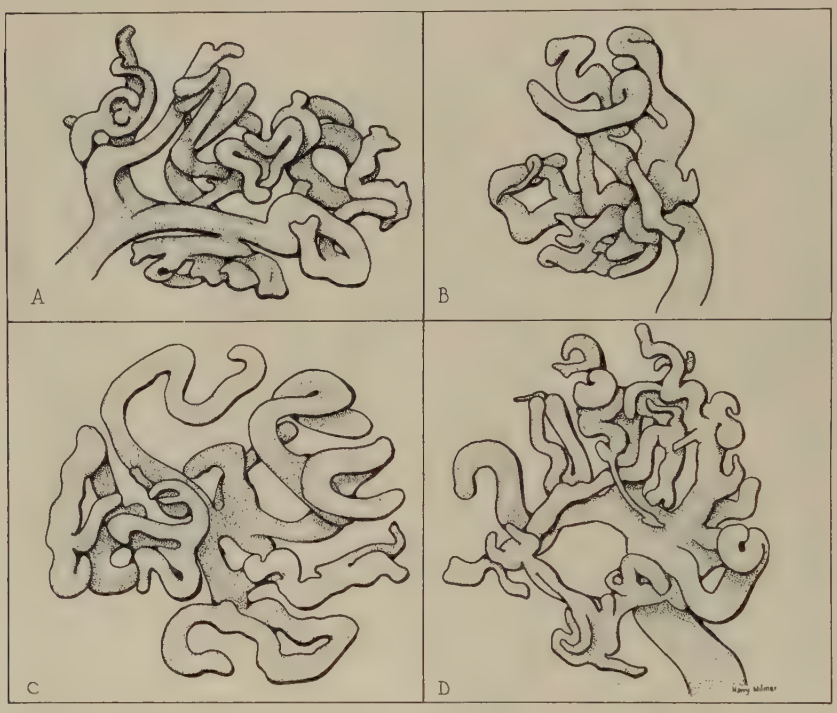

Fig. 3 Four partially injected glomeruli showing the primary branches and the beginning of the secondary branches. A, B, and D are viewed from the lateral aspect and C is viewed from above.

there is occasionally a diminution in the size of the vessels where the injection stopped (fig. 1, A). This is probably due to inherent factors in the injection technic since completely injected specimens show the vessels to be of more nearly uniform caliber (fig. 1, B).

While the termination of these secondary vessels could not be determined, it would appear that, if anastomoses had existed between the afferent and efferent vessels or between

the primary or secondary divisions, it would seem that they would have been injected like any other vessels. In the former case, a cast of the efferent arterioles would have been obtained before the glomerulus was completely injected, something which was never seen.

Since Johnston's reconstruction was made on a child of 3 months, the writer injected newborn kidneys with the thought in mind that anastomoses might exist early and be occluded

4

5

Fig. 4 Partially injected glomerulus showing many secondary branches arising at right angles from the primary vessels.

Fig. 5 Partially injected glomerulus showing very tortuous primary branches bearing very few secondary branches up to the point of injection. The primary branches are located at the periphery. The afferent arteriole divides into two large branches, each bearing a non-anastomosing group of smaller vessels.

later, but these specimens showed the same pattern as in the adult.

Ellinger in 1929 observed that the branches of the afferent arteriole were located at the periphery of the lobules and the efferent arterioles toward the center; he concluded that when there was a rise in pressure in the afferent arteriole the peripheral capillary loops would be pressed apart and more space made for the efferent channels in the interior.

Likewise, he noted that with an increased pressure in the efferent arteriole the peripheral vessels would be compressed and the flow through these vessels impeded. Ellinger thus concluded that in acute active hyperemia flow from the tuft is assisted, whereas in passive congestion, pressure on the capillaries blocks circulation. Our anatomical observations are compatible with such an hypothesis.

The figure of the completely injected glomerulus (fig. 1, B) shows the general arrangement of the peripheral vessels. The specimens are too small to permit following the vessels microscopically towards the center of the lobule where they unite with the efferent arterioles. Nevertheless, they do show graphically the superficial pattern of the capillaries and the lack of peripheral anastomoses which, indeed, were never seen in any specimen. The capillaries or secondary branches present a serpiginous course, some running for a comparatively long distance and exposing a relatively great surface area.

CONCLUSIONS

By the celloidin corrosion injection technic the branching of the glomerular tuft has been observed in hundreds of partially and completely injected glomeruli. While previously the glomeruli have been examined and reviewed by numerous observers, the writer has sought to present in permanent form pictures of actual specimens.

As revealed by injection methods the afferent arteriole branches into primary and corresponding secondary branches. Approximately the first two-thirds of the afferent arteriolar system can be carefully observed by injections exhibiting various degrees of penetration. When glomeruli are more completely injected they become too complicated to observe accurately under the microscope. The periphery of completely injected glomeruli, however, can be observed accurately. In these two portions (the major course of the primary and secondary vessels, and the peripheral capillaries in the glomerular tuft) the writer has never seen anastomoses in the hundreds of such glomeruli studied.

By the technique used the writer was unable to follow the finer vessels below the peripheral capillaries. These finer vessels have, however, been carefully studied by Vimtrup ('28) who was unable to find any anastomoses. In the human glomerulus there are probably no anastomoses.

In this drawing of the completely injected glomerulus it is believed that the general arrangement of the superficial pattern of the adult human glomerulus has been displayed graphically for the first time.

ACKNOWLEDGMENT

I am greatly indebted to Dr. E. T. Bell for suggesting this study, for the opportunity to execute it and for indispensable advice and criticism.

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REVIEWS OF APPROVED FILMS IN ANATOMY AND BIOLOGY

The following films in biology have been approved by one or more members of the Committees of the American Association of Anatomists or the American Society of Zoologists, and The Wistar Institute Committee.

Committee of the American Association of Anatomists:
Eliot R. Clark, Warren H. Lewis, Carl C. Speidel, and consultants.

Committee of the American Society of Zoologists:
Ralph Buchsbaum, Robert Chambers, Oscar W. Richards, and consultants.

Committee of The Wistar Institute of Anatomy and Biology:
Edmond J. Farris, Cranford Hutchinson, John S. Nicholas, and others as appointed.

NORMAL AND ABNORMAL WHITE BLOOD CELLS IN TISSUE CULTURE

WARREN H. LEWIS AND MARGARET R. LEWIS

(400 feet, monochrome, silent)

This film shows the various types of normal leucocytes in movement, bringing out strikingly the differences, in mode of progression, between neutrophiles, eosinophiles, lymphocytes, monocytes, myelocytes and myeloblasts. It shows, further, the behavior of white blood cells taken from a variety of abnormal conditions, such as lymphoid leukaemia, lymphoblastic sarcoma, and monocytic leukaemia. For good measure, there are beautiful pictures of epitheloid cells and the "Langhans" type of giant cell both from the spleen and from tubercles.

Photography, legending, length of scenes, and choice of subjects leave nothing to be desired. The film should be of great value both for research and teaching. ★A — Eliot R. Clark.

MECHANISMS OF BREATHING

ANTON J. CARLSON AND VICTOR JOHNSON

(400 feet, monochrome, sound)

Distributor: Erpi Classroom Films Inc.

This picture reveals mechanism of breathing by means of animation and some natural photography. Technical animation portrays the respiratory system and muscular activity involved in breathing, gaseous exchange in the lungs and body tissue cells with reference to pathological conditions, the nervous control of breathing and

factors affecting the rate and depth of breathing. The natural photography includes a bell-jar demonstration showing action of animal lungs and diaphragm action, capillary circulation and artificial respiration.

This sound picture is recommended for elementary and undergraduate college students. B — E. J. Farris.

CONGENITAL ANOMALIES

JACOB SARNOFF

(400 feet, monochrome, silent)

Living children are shown with the following anomalies: meningoencephalocele, spina bifida, imperforate anus, exstrophy of the bladder, club-foot, pseudo-hermaphroditism, hypospadias, exstrophy of liver, spleen and intestine, Hirschsprung's disease, supernumerary digit, hare-lip, and two unlabelled anomalies, one apparently an esophageal obstruction and the other some form of small mouth tumor. The various anomalies are well shown, and the film should be useful as a supplement to a course in human embryology or pathology in which congenital anomalies have been studied. The effectiveness of the film would be improved by descriptive legends, or better still, by the addition of a sound-track. D — Eliot R. Clark.

THE MANAGEMENT OF CICATRICIAL STRICTURE OF THE ESOPHAGUS

E. H. INGERSOLL

(400 feet, color, silent)

This film depicts principally the treatment of stricture of the esophagus; but there is also included a scene showing the treatment of bronchiectasis by blowing sulphanilamide into the bronchi with the aid of the bronchoscope.

The esophageal strictures are treated by the passage of sounds guided by threads. In the most severe case the thread is drawn through a gastrotomy opening; in the other cases the thread is swallowed and permitted to anchor itself in the coils of the intestine.

The story is well told, photography is excellent, length of scenes satisfactory, and legends clear and fully explanatory. The film should be of value in the teaching of surgery. ★E — Eliot R. Clark.

★ Copy deposited in the Film Depository of The Wistar Institute, and is available to all scientists for use at The Wistar Institute.

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